A 70-kDa Apolipoprotein Designated ApoJ Is a Marker for Subclasses of Human Plasma High Density Lipoproteins*

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A new apolipoprotein, termed apolipoprotein J (apoJ), was purified from human plasma by immunoaffinity chromatography. ApoJ is a glycoprotein consisting of disulfide-linked subunits of 34–36 and 36–39 kDa. Each subunit is glycosylated and has a pI range of 4.9–5.4. ApoJ exists in the plasma associated with high density lipoproteins (HDL) and specifically with subclasses of HDL, which also contain apoAI and cholesteryl ester transfer protein activity. Immunoaffinity purified apoJ-HDL subclasses have apparent molecular masses of 80, 160, 240, 340, and 520 kDa, as determined by gradient gel electrophoresis. By negative staining electron microscopy, apoJ-HDL range in diameter from 5 to 16 nm. Fractionation of plasma by vertical gradient density centrifugation revealed apoJ-HDL in HDL2 (d 1.063–1.125 g/ml) with the majority overlapping HDL3 (d 1.125–1.21 g/ml) and very high density lipoprotein (d 1.21–1.25 g/ml). The bimodal density distribution of apoJ-HDL suggests that these subclasses have a unique metabolic relationship and may play a role in the transport of cholesterol from peripheral tissues to the liver.

Plasma high density lipoproteins (HDL)† have received significant attention recently due to the correlation between decreased HDL-cholesterol and HDL-apoAI and increased risk of premature coronary artery disease (1–5). This correlation can explain, in part, why obesity, diabetes mellitus, hypertriglyceridemia, sedentary lifestyle, and maleness also can predict premature artery disease (6) since these conditions are associated with low HDL-cholesterol. The major physiological role postulated for HDL, consistent with its inverse association with disease, is to mediate reverse cholesterol transport (7–9). In this role, HDL collect unesterified cholesterol from tissues, facilitate its esterification by lecithin:cholesteryl acyltransferase (LCAT) which utilizes apoAI as an activating cofactor (10), serve as donors of cholesteryl esters (CE) to other lipoproteins in reactions catalyzed by the cholesteryl ester transfer protein (CETP) (11), and deliver CE to the liver for excretion. HDL can also supply functional apolipoproteins to other lipoproteins, specifically apoCII to chylomicrons and very low density lipoproteins in the plasma for activation of lipoprotein lipase (12) and apoE to chylomicrons in the intestinal lymphatics (13).

Our understanding of HDL metabolism is surprisingly limited. Depending on the method of isolation, at least eight discrete HDL subclasses have been identified (14, 15). The metabolic interrelationship between the subclasses is not well established nor have specific functions been assigned to the different subclasses. It may be that different HDL subclasses are targeted to different tissues. The irreversible uptake of HDL by tissues has been studied in animal models, particularly the rat (16). The important sites of HDL delivery are the liver and steroidogenic tissues. HDL-cholesterol appears to enter cells by receptor-mediated (17, 18) and receptor-independent pathways (19–21). Even the origin of HDL particles is uncertain. Nascent HDL are synthesized both in the liver and intestine (22–24). In addition, surface constituents released during the catabolism of triglyceride-rich lipoproteins can contribute to one or all of the HDL subclasses (25–27).

Ellsworth et al. (28) and Hopkins et al. (29) presented evidence that CETP activity is necessary for the speciation of HDL and particularly that which occurs as a consequence of the hydrolysis of very low density lipoprotein-triglycerides catalyzed by lipoprotein lipase (28). The normal distribution of HDL subclasses is markedly altered in patients with complete deficiency of CE transfer activity (30). A role for CETP in HDL speciation is consistent with its capacity to bind to HDL (31). The HDL with which CETP is associated may be the preferred substrates from which CE is transferred to other lipoproteins. The rate-limiting step in the lipid transfer reaction is the formation of a ternary complex comprised of CETP and the donor and acceptor lipoproteins (32). If CETP is already complexed with HDL, the transfer process is favored (32). Cheung and colleagues (33) reported that, in vivo, CETP is associated with specific subclasses of HDL which contain apoAI but not apoAI1. Francone et al. (34) determined that the HDL containing apoAI and a substantial portion of...
the CETP activity involve only about 2% of the total plasma apoAI and can also contain apoD and LCAT activity. These findings lend credence to the hypothesis that the CETP-HDL interaction is physiologically relevant.

The apparent association of CETP with specific populations of HDL raises the possibility that CETP activity is regulated by lipoprotein constituents. In fact, the accumulation of lipolytic products (35) and cholesterol (36) at the lipoprotein surface can, respectively, enhance and retard CETP-catalyzed lipid transfer. Apolipoproteins may also regulate CETP activity (37, 38). We assume that the activity of CETP is controlled by the lipid and apolipoprotein constituents with which it associates, to achieve a more complete understanding of CETP and to identify apolipoproteins which are associated with it, we used an immunochromatographic approach. Monoclonal antibodies were raised against partially purified CETP. These antibodies were screened to select those which recognized CETP and those which recognized CETP-associated proteins. Such a reagent is of great value for obtaining insight into the mechanism of transfer activity (37, 3.8). We assume that the activity of CETP is controlled by the lipid and apolipoprotein constituents with which it associates.

EXPERIMENTAL PROCEDURES

Materials—Polyacrylamide gel reagents, nitrocellulose paper, and secondary antibodies for monoclonal antibody characterization and electroimmunoblotting were obtained from Bio-Rad or from Iles-Yeda. The MonoQ anion-exchange column, gradient media, high molecular weight protein standards, and ampholytes were obtained from Pharmacia LKB Biotechnology Inc. Prestained protein standards were purchased from Bethesda Research Laboratories. Trifluoroacetic acid was purchased from Pierce Chemical Co. and HPLC grade phosphoric acid was purchased from Sigma. Nuclear were [9, 10-3H]triolein ([3H]TG, 26.8 Ci/mmol) and [cholesterol-4,5-3H]cholesterol ([1H]TC, 26.8 Ci/mmol) and [cholesteryl-

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pH 7.4, 150 mM NaCl, 5% nonfat dry milk, 0.01% (v/v) antifoam A, and 0.001% (w/v) merthiolate. The blots were washed thoroughly in blocking buffer, incubated for 2 h with horseradish peroxidase-conjugated secondary antibody (1:5000), and developed by the method of Towbin et al. at 46.

Two-dimensional Gel Electrophoresis—Isoelectric focusing gels, pH range 4-8, were cast in glass tubes (3 mm x 11 cm) as described by O’Farrell (47) and modified by Anderson and Anderson (48). MAb11 eluate was solubilized (9.5 M urea, 5% -mercaptoethanol, 2% SDS, 10% glycerol, 5% ampholines, and 3% Nonidet P-40) for 30 min, and electrophoresed at 400 V for 12 h. The extruded gels were placed on a Laemmli polyacrylamide gel (10%) (45) and electrophoresed at 35 mA/gel for 5.5 h. Proteins were stained with CBB or transferred to nitrocellulose as described above.

Native Gradient Gel Electrophoretic (GGE) Analysis—MAb11 eluate and HDL (1.063–1.21 g/ml) were subjected to nondenaturing gradient gel electrophoresis, using Pharmacia 4/30 gradient gels as specified by the manufacturer. Samples were adjusted to 10% glycerol and electrophoresed for 24 h at 10°C. The gels were stained with CBB or the protein was transferred to nitrocellulose for immunostaining.

Lipids were stained with Sudan Black, as described by Pratt and Dangerfield (49). To analyze the protein components of the lipoproteins resolved by GGE, protein was electroeluted overnight at 10°C in 100 mM Tris, pH 9, containing 0.1% sodium dodecyl sulfate, using an Isoe electrolution apparatus. ApoA1 and apoA2 were detected by electroimmunoblotting.

Single Spin Density Ultracentrifugation of Human Plasma—Blood drawn from fasted donor was density-adjusted with solid KBr and centrifuged in a fixed angle rotor as described by Chung and colleagues (50). Procedure 15 was used in which the density-adjusted plasma was centrifuged in a discontinuous gradient for approximately 4.3 h; 1-ml fractions (35 total) were collected. Each fraction was assayed for apoA1 and apoA2 by EIA. Protein (0.5 mg) in 100 µl of 10 mM NH₄HCO₃ was added to wells of 96-well microtiter plates. The EIA was conducted as described above except that the temperature for blocking and antibody incubation was 25°C. Primary apoA1 or polyclonal apoA1 antibody in blocking buffer was used at 1:1000 and 1:10000 dilution, respectively.

Electron Microscopy of Negatively Stained Lipoproteins—HDL (1.063–1.21 g/ml) were isolated from plasma obtained from a normolipemic donor (12). Immunoaffinity purified apoA1-containing lipoproteins (mAbl1 eluate), eluted from mAb11-Affi-Gel with 1 M HOAc, were dialyzed into 10 mM NH₄HCO₃. Both HDL (114 µg/ml) and the mAb11 eluate (80 µg/ml) were prepared for electron microscopy by the method of Forte and Nordhausen (51). Briefly, samples were diluted 1:1 in 2% phosphotungstic acid, pH 7.4, and 5 µl were deposited on a Formvar-carbon-coated grid and allowed to air dry. Samples were examined with a Zeiss EM10 transmission electron microscope.

Analytical Methods—Phospholipid was quantitated by the method of Bartlett (52). Cholesterol and triglyceride were determined as specified by the Sigma kits. Protein concentrations were assayed relative to a bovine serum albumin standard, using the method of Lowry et al. (53), modified by the addition of 1% SDS, or by the bichinchoninic acid protein determination assay (Fierce Chemical Co.). Protein sequence was determined with an Applied Biosystems 470A gas phase sequencer; phenylthiohydantoin-derivatized amino acids were analyzed on a Waters HPLC, using a NOVA-PAK C₁₈ reverse-phase column.

RESULTS

A Monoclonal Antibody Raised against Partially Purified CETP Recognizes a 70-kDa Plasma Protein—A preparation of CETP purified 3500-fold (39) was used to raise monoclonal antibodies to characterize CETP and associated proteins. The antigen was comprised of a major protein of 67–70 kDa, a protein of 58 kDa and minor proteins, as determined by SDS-PAGE. The 67–70-kDa protein was distinct from albumin and had CETP activity when electroeluted from the gel (54). Since CETP was the catalytic protein of interest in any complexes containing it, the isolation of CETP mAb was the highest priority. Antibodies which reacted with partially purified CETP, identified by EIA, were initially chosen. Positive clones were subsequently screened by electroimmunoblotting of plasma and partially purified CETP, and the few mAb which recognized the 67–70-kDa protein were selected with the expectation that these were CETP mAb. A highly reactive positive clone, designated clone 11 and producing an IgG, mAb11, was expanded and subcloned at limiting dilution. Antibody from all subclones reacted identically to the parent clone, as assessed by EIA and electroimmunoblotting, and the clonality of the antibody secreted by the parent clone was confirmed by two-dimensional gel electrophoresis.

The reactivity of mAb11 toward proteins in human plasma and in partially purified CETP, determined by electroimmunoblot analysis, is illustrated in Fig. 1. Under nonreducing (NR) conditions, mAb11 recognized a 70-kDa protein in plasma (panel A) and in partially purified CETP (panel B). In the presence of 20 mM DTT (reduced, R), the protein recognized by the antibody migrated as a heterogeneous band at ~35 kDa. Based on electroimmunoblot analysis, the protein (70(NR)/35(R))-kDa protein was also present in CETP preparations purified 15,000-fold (not shown). Human serum albumin was not recognized by mAb11. CETP activity has been reported to be associated both with 70- (40) and 35-kDa (55) proteins, and it is generally accepted (see, for example, Ref. 40) that plasma contains several lipid transfer catalysts which may or may not be related. Thus, these results suggest that mAb11 recognizes CETP.

MAb11 Removed CE and TG Transfer Activity from Plasma—To determine if mAb11 removes CETP from plasma, plasma was chromatographed on a mAb11 affinity column. Both the 70(NR)/35(R)-kDa protein recognized by mAb11 and CE and TG transfer activities were retained on the column and were eluted in 3 M KSCN. The eluate was termed mAb11 eluate. The results of a typical lipid transfer assay of mAb11 eluate are illustrated in Fig. 2. In 15 h, 20 µg of mAb11 eluate protein catalyzed the transfer of 5.6% of the [14C]CE and 3.2% of the [3H]TG from radiolabeled LDL to unlabeled HDL. The neutral lipid transfer of the eluate was concentration-dependent and varied with the time of the assay. For Fig. 2, the mAb11 eluate-mediated transfer of CE and TG was determined in the HDL-acceptor, LDL-donor assay in which the lipoproteins were separated by precipitation. These results were corroborated by separation of lipoproteins by ultracentrifugal flotation. The transfer of neutral lipids facilitated by mAb11 eluate also occurred from HDL to LDL.

MAb11 Does Not Inhibit CE or TG Transfer Catalyzed by

Fig. 1. MAb11 detects a nonreduced 70 kDa and a reduced 35-kDa antigen in plasma and in partially purified CETP. Human plasma (panel A, 60 µg) or partially purified CETP (panel B, 10 µg) were solubilized in the absence (nonreduced, NR) or presence of 20 mM DTT (reduced, R). The samples were heated at 55°C for 30 min, loaded on an SDS-polyacrylamide "mini" gel comprised of a 4% acrylamide stacking and 10% acrylamide resolving portion, and electrophoresed at 20 mA for 1.5 h. The proteins were transferred to nitrocellulose for electroimmunoblotting with mAb11.

RESULTS
CETP—The ability of mAbl1 to remove CETP activity from plasma supported the possibility that mAbl1 is an antibody specific for CETP. To test this hypothesis further, we asked whether mAbl1 could inhibit CETP activity. Increasing concentrations of mAbl1 were added to a transfer reaction consisting of radiolabeled LDL, unlabeled HDL, and CETP, purified 15,000-fold. To establish that CETP could be inhibited by antibodies, we showed that the polyclonal antibody raised against partially purified CETP (panel A) removed transfer activity in a concentration-dependent manner (Fig. 3, panel A). Addition of similar concentrations of preimmune goat immunoglobulin had no effect. In contrast, direct addition of mAbl1 had no effect on the catalyzed transfer of CE or TG (not shown). Moreover, mAbl1 failed to remove CE or TG transfer activity (panel B) when a high affinity goat antimouse IgG secondary antibody was included with mAbl1 to achieve immunoprecipitation. The mAb from the secreting myeloma P3X63Ag8 used as a control in the indirect immunoprecipitation experiments had no effect on CE or TG activity. To confirm that the 70(NR)/35(R)-kDa protein was removed from the CETP preparation by mAbl1, the precipitates were collected by centrifugation and the 70(NR)/35(R)-kDa protein was detected by electroimmunoblotting. Although mAbl1 removed the 70(NR)/35(R)-kDa protein without removing transfer activity, there was a consistent and reproducible increase in TG transfer which occurred at low antibody concentrations. Removal of the 70(NR)/35(R)-kDa protein by indirect immunoprecipitation without concomitant removal of transfer activity indicates that the 70(NR)/35(R)-kDa protein is not CETP or another protein with CE and TG lipid transfer capability. Moreover, the data suggest that it is not an inhibitor of neutral lipid transfer.

The mAbl1 Eluate Contains the 70(NR)/35(R)-kDa Protein and apoA1—The finding that the antigen recognized by mAbl1 was not CETP raised two questions. Has the 70(NR)/35(R)-kDa protein been described previously and is it an apolipoprotein? If it is an apolipoprotein, the mAbl1 eluate should consist of lipid and perhaps other apolipoproteins. To address these questions, mAbl1 eluate was obtained for structural studies. Fresh human plasma, diluted in buffer containing protease inhibitors, was chromatographed on mAbl1-Affi-Gel and on a control Affi-Gel column to which no antibody was attached. The resulting eluates were analyzed by SDS-PAGE (Fig. 4). Panel A illustrates the CBB-stained gel of the eluate from control-Affi-Gel (lanes 1 and 2) and from mAbl1-Affi-Gel (lanes 3 and 4). No protein was detected by CBB in the eluate from the control Affi-Gel column. In the absence of reductant (NR) (lane 3), the mAbl1 eluate consisted of two major proteins of 70 and 28 kDa. The migration of the 70-kDa protein changed upon reduction (R) (lane 4), resulting in multiple, closely spaced bands at ~35 kDa. The migration of the 28-kDa protein was not altered by reduction (lanes 3 versus 4).

Panels B and C are electroimmunoblots of samples identical to those in lanes 1–4 (panel A) but stained with mAbl1 (panel B) or apoAI mAblB7 (panel C), respectively. The 70-kDa protein and its reduced forms at 35 kDa reacted with mAbl1 (panel B, lanes 7 and 8, respectively). The mAbl1 immunoreactivity which remained at 70 kDa in the presence of reductant was not affected by alkylation, suggesting that a fraction of the 70-kDa protein was resistant to reduction. Since the 70-kDa protein was not visible in the CBB-stained gel under reducing conditions, its contribution to the total 70-kDa species was small. The 28-kDa protein which was present in the mAbl1 eluate but not in the control eluate was apoA1, identified by electroimmunoblotting with mAblB7 (panel C, lanes 3–4).

FIG. 2. Chromatography of plasma on a mAbl1-Affi-Gel column removes neutral lipid transfer activity. Eluate from the mAbl1 column, obtained with 3 M KSCN, was assayed for CE (●—●) and TG (▲—▲) transfer activity, using $[^{3}H]$TG-[^{14}C]CE-labeled LDL and unlabeled HDL and an incubation time of 15 h, as described under "Experimental Procedures." Background transfer which occurred in the absence of catalyst was subtracted.

FIG. 3. mAbl1 does not inhibit neutral lipid transfer activity. Purified CETP (15,000-fold, 1 μg) was incubated overnight at 4°C with varying amounts of purified goat IgG raised against partially purified CETP (1 mg/ml) (panel A) or purified mAbl1 (0.7 mg/ml) (panel B) in 50 mM Tris-HCl, pH 7.4. Antigen-antibody complexes were removed by centrifugation as described under "Experimental Procedures," and the supernatants were assayed (3 h at 37°C) for CE (●—●) and TG (▲—▲) transfer, using radiolabeled LDL and unlabeled HDL. Preimmune goat immunoglobulin and a purified nonrelated IgG, from the secreting myeloma P3X63Ag8 had no effect on the transfer of TG or CE. The data are represented as percent of untreated controls.

FIG. 4. ApoAI is present in mAbl1 eluate. Control and mAbl1 eluates were prepared by chromatographing 25 ml of human plasma, diluted 1:3 in phosphate-buffered saline containing protease inhibitors, on Affi-Gel (control) or mAbl1-Affi-Gel as specified under "Experimental Procedures." Bound protein was eluted in 50 mM TEA and dialyzed immediately into 10 mM NH₄HCO₃. Equal volumes (1 ml for stained gel analysis and 100 μl for electroimmunoblot analysis) of each eluate were lyophilized, solubilized, and electrophoresed for 5.5 h at 30 mA. The proteins were stained with CBB (panel A) or transferred to nitrocellulose and incubated with apoAI mAbl1 (panel B) or apoAI mAblB7 (panel C). The absence or presence of 20 mM DTT is denoted as nonreduced (NR) or reduced (R), respectively.
and apoAI, a marker of HDL. In other studies, mAb11 eluate was also found by electroimmunoblot analysis to contain very minor amounts of apoII, apoAI, apoE, and apoD; however, no apoB or LCAT was detected. The 70(NR)/35(R)-kDa protein recognized by mAb11 was designated apolipoprotein J. The apolipoprotein designation was justified by the fact that mAb11 eluate also contained lipid. MAb eluate was 10.6 ± 1.8% lipid as: 6.3 ± 1.4% phospholipid, 3.3 ± 0.3% cholesterol, and 1.0 ± 0.3% triglyceride (n = 3). Negligible apoJ or apoAI bound to control-Affi-Gel which indicates that apoJ and apoAI were specifically removed from plasma by mAb11. The method of elution of the columns, using TEA, HOAc, or KSCN, did not influence the results. The amount of mAb11 eluate protein typically obtained from 25 ml of plasma, under conditions where >90% (n = 3) of the mAb11 immunoreactivity was removed, was 1.7 mg, about 80% of which was apoJ based on relative CBB staining intensity. Therefore, we estimate that the plasma apoJ concentration is ~9 mg/dl.

**ApoJ Consists of Two Subunits—Electrophoretic analysis of mAb11 eluate** (Fig. 4, panel A, lane 4) indicated heterogeneity of the 35-kDa subunits of apoJ. To provide additional information about the heterogeneity, mAb11 eluate was analyzed by two-dimensional gel electrophoresis under reducing conditions; the results are shown in Fig. 5. The 35-kDa component consisted of two proteins, each with a pI of 4.9–5.4 (left panel). The larger of the two was 36–39 kDa, and the smaller was 34–36 kDa. MAb11 recognized both proteins (right panel). Consistent with results shown in Fig. 4 (panel B, lane 8), mAb11 detected a 70-kDa protein, in addition to the 35-kDa species, after reduction which was not detected by CBB (Fig. 5, left panel). The pI of apoAI, the 28-kDa protein in mAb11 eluate, was 5.6, as reported (57). MAb11 did not recognize two other additional proteins, a 90-kDa protein with a pI of 6.4–6.9 and a 45-kDa protein with a pI of 5.6. These proteins were not characterized further in this study.

**MAb11 Eluate Contains Distinct Lipoprotein Species**—The presence of both apoAI and lipid in the mAb11 eluate suggested the existence of macromolecular complexes. To assess this possibility, the eluate was analyzed by nondenaturing GGE (Fig. 6). Panel A of Fig. 6 shows a CBB-stained gel of mAb11 eluate compared with HDL (d 1.063–1.21 g/ml), isolated by sequential ultracentrifugation, for reference. HDL consisted of lipoproteins which ranged in size from 100 to 400 kDa, consistent with the sizes of ultracentrifugally isolated HDL reported by Blanche et al. (58). MAb11 eluate consisted of four major species of 80, 160, 240, and 340 kDa and a minor component of 520 kDa. The relative distribution of these particles, determined by densitometric scanning, was 80 kDa, 35%; 160 kDa, 8%; 240 kDa, 17%; 340 kDa, 27%; 520 kDa, 13%. The sizes and the distribution of particles in mAb11 eluate are consistent with the size distribution of immunofinity purified HDL analyzed by GGE (59). An identical gel stained with Sudan Black (panel B) revealed that the 160-, 240-, and 340-kDa complexes contained lipid. If lipid was present in the 80- and 520-kDa complexes, it was below the level detectable by this assay. All sizes of complexes contained apoJ, as determined by electroimmunoblotting (panel C). Using mAbB7 (panel D), apoAI was revealed in the 160-, 240-, and 340-kDa complexes; low levels of apoAI immuno-reactivity were also detected in the 80- and 520-kDa complexes.

For Fig. 6, mAb11 eluate was obtained with TEA. The results were the same when HOAc was used to elute bound protein from the column. Furthermore, a comparison of fresh plasma and mAb11 eluate by nondenaturing GGE coupled with antiapoJ immunostaining revealed that the sizes of apoJ-lipoproteins in plasma and in mAb11 eluate were the same. This result indicates that elution of apoJ-lipoproteins from the immunofinity column did not alter their sizes.

The proteins of the mAb11 eluate complexes resolved by nondenaturing GGE were also evaluated by SDS-PAGE to confirm the presence of apoJ and apoAI. The complexes, designated 1–5 (Fig. 6, panel A), were electroeluted and subjected to SDS-PAGE in the presence of DTT followed by electroimmunoblot analysis. The samples shown in the left panel of Fig. 7 were stained with mAb11, and the results establish that complexes 1–5 contained apoJ of the expected reduced size of ~35 kDa. Electroimmunoblot analysis with apoAI mAbB7 (Fig. 7, right panel) confirmed that apoAI, the 28-kDa protein, was also present in each complex. HDL (d 1.063–1.21 g/ml) were also evaluated for the presence of apoJ and apoAI by electroimmunoblotting. ApoJ was not detected in these HDL which had been isolated by sequential ultracentrifugal flotation. As expected, apoAI was detected (Fig. 6, panel D; Fig. 7, right panel).

**MAb11 Eluate Contains Lipoproteins Which Resemble HDL**—Freshly prepared HDL, isolated by sequential ultracentrifugal flotation, and mAb11 eluate were stained with

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**Fig. 5. Two-dimensional gel analysis of mAb11 eluate.** MAb11 eluate (30 µg), obtained as detailed in Fig. 4, was analyzed by two-dimensional gel electrophoresis in the presence of 5% β-mercaptoethanol as described under “Experimental Procedures.” Proteins were stained with CBB or were transferred to nitrocellulose and incubated with mAb11.

**Fig. 6. The mAb11 eluate contains lipoproteins which are similar to HDL in size.** HDL (d 1.063–1.21 g/ml; 47 µg for CBB stain and 132 µg for Sudan Black stain) or mAb11 eluate (47 µg for CBB stain and 132 µg for Sudan Black stain) in 10 mM NH_4HCO_3, were adjusted to 10% glycerol, loaded on 4–30% nondenaturing gradient gels, and electrophoresed for 24 h at 125 V. MAb11 eluate was obtained in 50 mM TEA. Protein was stained with CBB (panel A) and lipid, with Sudan Black (panel B). For electroimmunoblots (panels C and D), mAb11 eluate (17 and 27 µg for apoJ and apoAI analysis, respectively) or HDL (155 and 15 µg for apoJ and apoAI analysis, respectively) resolved by GGE and transferred to nitrocellulose, were incubated with apoJ-specific mAb11 (panel C) and apoAI-specific mAbB7 (panel D).
HDL Subfractions Contain Apolipoprotein J

13245

HI

12345+ 12345+

kDa lil- -h L

68-

45-

30-

18- mAbl1 mAbB7 IaapoAIl

FIG. 6. SDS-PAGE and electroimmunoblot analysis of protein components present in mAbl1 eluate complexes resolved by GGE. The complexes in mAbl1 resolved by GGE and designated 1–5 in Fig. 6, were electroblotted, electrophoresed, and electroimmunoblotted as described under “Experimental Procedures.” For comparison, HDL (d 1.063–1.21 g/ml; 10 µg for mAbl7 or 50 µg for mAbl1 staining) were also electrophoresed and electroimmunoblotted. Left panel, electroimmunoblot with apoJ mAbl1; right panel, apoAl mAbl7.

mAb11 eluate

HDL

Fig. 8. Comparison of mAbl1 eluate and HDL by electron microscopy. HDL (d 1.063–1.21 g/ml; 114 µg/ml) and mAbl1 eluate (80 µg/ml), obtained in 50 mM TEA, were stained with phosphotungstic acid and visualized by electron microscopy (Fig. 8). The size of the particles in each sample was estimated by measuring the diameters of 100 particles in a representative field of each micrograph. MAbl1 eluate (Fig. 8, top) consisted of spherical particles which ranged in diameter from 5 to 16 nm. Of these, approximately 50% were 5–9 nm, 30% 9–12 nm, and 20% 12–16 nm. Of the particles, 70% were <10 nm and 30% were >10 nm in diameter. The size distribution of particles in mAbl1 eluate agrees well with the range of 5–16 nm reported for HDL isolated by immunoaffinity chromatography (60). HDL isolated by immunoaffinity chromatography have a broader size range than those isolated by ultracentrifugal flotation have a broader size range than those isolated by ultracentrifugal flotation (60 versus 81). The HDL which we isolated by ultracentrifugal flotation ranged from 5 to 12 nm in diameter (Fig. 8, bottom); approximately 45% were 5–9 nm, 45% 9–12 nm, and 10% 12–16 nm.

Comparison of the Distribution of apoAl and apoJ in Human Plasma—Plasma from two donors (female and male) was rapidly fractionated in a discontinuous salt gradient (50) to minimize disruption of lipoprotein particles relative to that which can result from sequential ultracentrifugal flotation in which long centrifugation times are used. The gradient, selected to allow optimal separation of the HDL subclasses HDL2 and HDL3, is indicated in panel A of Fig. 9. These particular donors were selected because they had comparable levels of plasma cholesterol (143 and 149 mg/dl, respectively) and triglyceride (59 and 70 mg/dl, respectively) but very different LDL/HDL ratios. The female donor had an LDL-cholesterol/HDL-cholesterol ratio of 1.0; the male donor an LDL-cholesterol/HDL-cholesterol of ratio 2.1. The relative distribution of apoAI and apoJ, as % of the protein in each fraction, was assessed by EIA. The profile of the female donor is shown in panel B and that of the male donor, in panel C. Peaks of apoJ occurred in both the HDL2 and HDL3 region, fractions which also contained apoAl. In the plasma from both subjects, apoJ was present in the HDL2 density range (d 1.063–1.125 g/ml), in the tailing edge of HDL3 (d 1.125–1.21 g/ml) and in the density range containing very high density lipoproteins (VHDL, d 1.21–1.25 g/ml). No apoJ was detected in fractions of d < 1.063 g/ml, but apoJ was also present in fractions of d > 1.25 g/ml. Within the density range of 1.063–1.25 g/ml, there were fractions which contained apoAI but no detectable apoJ, supporting the GGE results which suggest that apoJ is associated with discrete subclasses of HDL.

DISCUSSION

This report establishes the existence of a unique protein, designated apoJ, which is associated with plasma lipoproteins. ApoJ is a 70-kDa disulfide-linked dimer of ~35-kDa subunits, both of which have pI values between 4.9 and 5.4. The
lipoproteins which contain apoJ are HDL. This conclusion is supported by two lines of evidence. First, apoJ and apoAI, a marker of HDL, are associated with the same sized particles in mAb11 eluate. Electrophoresis of mAb11 eluate on gradient gels under non-denaturing conditions resolves four major and one minor discrete subclasses of lipoproteins. apoJ and apoAI exist within all five subclasses. The molecular masses of the major subclass are within the range reported for HDL, 80–340 kDa. The minor subclass of 520 kDa is larger than typical HDL. Second, electron microscopic evidence indicates that mAb11 eluate contains spherical particles with diameters in the HDL range, 5–16 nm. The majority of the apoJ-containing HDL are small and dense, as determined by GGE and by discontinuous density gradient ultracentrifugation, suggesting that they can be classified as HDL$_2$ and VHDL. Taken together, the data support a bimodal density distribution of apoJ-HDL with the greatest percentage of apoJ-HDL in the HDL$_2$/VHDL classes.

Weighed against the evidence that apoJ defines distinct subclasses of HDL is the puzzling result that no apoJ was detected in HDL by electroimmunoblot analysis. The amount of apoJ in plasma is in the range of that of apoE and the apoCs. The abundance of apoJ, predicted on the basis of its yield from immunoaffinity chromatography of plasma samples isolated from three donors, is about 9 mg/dl. The range of apoJ determined in preliminary experiments for more than 41 donors, using a quantitative EIA, is 7–19 mg/dl. If most of the apoJ in plasma were in HDL, apoJ should have been detected in HDL by electroimmunoblotting, but was not. Since the HDL used in this study were isolated by sequential ultracentrifugal flotation, it is possible that apoJ (like apoAI, apoAI, apoB, and apoE) interconverts among HDL particles. apoJ is the apoJ-HDL, based on the amount of apoA1 associated with the subclass comprised of apoJ and apoAI; HDL subclasses which contain apoJ may account for only 2% of the HDL, based on the amount of apoAI associated with them. Although low in abundance, these HDL subclasses may have an important physiologic function. Among apolipoproteins in HDL, apoJ is unique in its molecular weight, subunit structure, and isoelectric point. The identification of apoJ in HDL presents exciting opportunities. According to current concepts, small HDL mature into large HDL as a result of cholesterol efflux and esterification processes. Thus, the apoJ-HDL in the HDL$_2$ density range may be products of the small dense apoJ-HDL. The apoJ-HDL in the HDL$_2$/VHDL density range have properties appropriate for cholesterol acceptors and LCAT substrates. They are small, poor in lipid, and contain the LCAT activator apoAI. Castro and Fielding (74) recently reported that small HDL, present in low abundance in plasma, preferentially accept effluxed cholesterol from cultured fibroblasts. Small HDL are also the preferred sites of LCAT action (75, 76). In fact, within the HDL$_2$ density range, the maximum velocity of the LCAT reaction correlates inversely with the size of the HDL$_2$ (76). The bimodal density distribution of apoJ-HDL suggests that an understanding of apoJ and apoJ-HDL subclasses may provide insight into the process of reverse cholesterol transport.

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