Clusterin (Complement Lysis Inhibitor) Forms a High Density Lipoprotein Complex with Apolipoprotein A-I in Human Plasma*

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Clusterin/human complement lysis inhibitor (CLI) is incorporated stoichiometrically into the soluble terminal complement complex and inhibits the cytolytic reaction of purified complement components C5b-9 in vitro. Using an anti-clusterin affinity column, we found that an additional protein component with a molecular mass of 28-kDa co-purifies with clusterin from human plasma. We show by immunoblotting and amino acid sequencing that this component is apolipoprotein A-I (apoA-I). By using physiological salt buffers containing 0.5% Triton X-100, apoA-I is completely dissociated from clusterin bound to the antibody column. Free clusterin immobilized on the antibody-Sepharose selectively retains apoA-I from total human plasma. Delipidated apoA-I and to a lesser extent ultracentrifugation-purified high density lipoproteins (HDL) adsorbed to nitrocellulose also have a binding affinity for purified clusterin devoid of apoA-I. The isolated apoA-I-clusterin complex contains approximately 22% (w/w) lipids which are composed of 54% (mole/mol) total cholesterol (molar ratio of unesterified/esterified cholesterol, 0.58), 42% phospholipids, and 4% triglycerides. In agreement with the low lipid content, apoA-I-clusterin complexes are detected only in trace amounts in HDL fractions prepared by density ultracentrifugation. In free flow isochromatography, the purified apoA-I-clusterin complex has the same mobility as the native clusterin complex in human plasma and is found in the slow-migrating HDL fraction of fasting plasma. Our data indicate that clusterin circulates in plasma as a HDL complex, which may serve not only as an inhibitor of the lytic terminal complement cascade, but also as a regulator of lipid transport and local lipid redistribution.

Human clusterin (synonymous names: SP-40,40; complement lysis inhibitor; or CLI; SGP-2; TRPM-2; apolipoprotein J)

1 is a 70-kDa glycoprotein found at concentrations of 50–100 μg/ml in normal human plasma and at about 10-fold higher concentrations in human seminal plasma (1–4). Initially, clusterin was identified in ram rete testis fluid as a major glycoprotein that mediates aggregation and clustering of a variety of homologous and heterologous cells in vitro (5). The serum form of sheep clusterin, as well as the deglycosylated testicular clusterin, however, lacked the cell-aggregating activity (6). Human clusterin isolated from blood and seminal plasma as well as rat seminal plasma clusterin, which is identical to the Sertoli cell-derived sulfated glycoprotein 2 (SGP-2), showed the same cell-agglutinating activity in vitro (2, 7).

Recently, clusterin was shown to be an integral constituent of the nonlytic soluble terminal complement complex (1, 8) which is formed by S-protein/vironectin and the components of the lytic complex C5b, C6, C7, C8, and C9. A monoclonal antibody raised against a contaminating component in S-protein preparations (9, 10) identified clusterin as an additional constitutive component of the terminal complement complex (1). Similarly, monoclonal antibodies raised against immune deposits in the kidney of glomerulonephritis patients (11) were shown to bind to clusterin (8), which co-localized with terminal complement complexes in those patients (12). The complete protein structure of human clusterin (1, 3, 4) has been determined by cDNA cloning and partial protein sequences are known for sheep (6) and hamster homologues (14). The protein precursor is post-translationally processed into two cysteine-linked 35-kDa subunits. The amino-terminal 76 residues display the typical features of an α-helical coiled-coil domain (1) and are followed by 22 residues (position 77–98) showing homology to the cysteine-rich thioredoxin type I modules in terminal complement proteins (3, 15).

Clusterin has also been characterized independently as TRPM-2 as one of those gene transcripts which are highly upregulated in regressing and involuting tissues (16, 17). Clusterin is induced in the prostate (16, 17) and in the androgen-dependent Shionogi mouse mammary tumor after androgen withdrawal (18), in the kidney in response to a pressure insult, in embryonic interdigital tissues that undergo programmed cell death, and in a mouse bladder tumor during chemotherapeutic regression (19). Increased mRNA levels have been observed in scrapie-infected hamster brain and in the hippocampus of patients with Alzheimer’s disease and Pick’s disease (14). A closely related quail protein called T64, of which 46% amino acid residues are identical to mammalian prostate message 2; C5b-9, the terminal complement complex; C5, C6, C7, C8 and C9, the fifth, sixth, seventh, eighth, and ninth component, respectively, of the complement system; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; ITP, isochromatography; TLC, thin layer chromatography; HPTLC, high performance TLC.

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§ The abbreviations used are: CLI, complement lysis inhibitor; SGP-2, sulfated glycoprotein 2; TRPM-2, testosterone-repressed prostate message 2; C5b-9, the terminal complement complex; C5, C6, C7, C8 and C9, the fifth, sixth, seventh, eighth, and ninth component, respectively, of the complement system; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; ITP, isochromatography; TLC, thin layer chromatography; HPTLC, high performance TLC.
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While purifying clusterin from fresh human plasma by monoclonal antibody-affinity chromatography, we observed that clusterin was consistently associated with an additional protein of human plasma. A partial amino-terminal sequence of this co-purifying protein was identical to that of apoA-I. In this report we have studied the interaction between clusterin and apoA-I in human plasma and demonstrate that clusterin forms a distinct type of HDL particles that may be involved in lipoprotein metabolism or lipid redistribution.

EXPERIMENTAL PROCEDURES

Purification of ApoA-I-Clusterin Complexes—Blood was obtained from healthy fasting volunteers. Cells and plasma were separated by low speed centrifugation at 4 °C in the presence of 10 mM EDTA. Then, plasma samples were diluted with four volumes of 100 mM NH₄OAc, 50 mM NaCl, pH 7.4, and loaded onto a 5-ml affinity column at 4 °C overnight. The column was washed extensively with 300 ml of 10 mM Tris-HCl, 10 mM EDTA, 500 mM NaCl, pH 7.4. The material was eluted with 0.2 M glycine, pH 2.5, by collecting 0.5-ml fractions which were immediately neutralized with a solution of Tris-HCl, pH 8.0. The eight fractions with the highest protein concentration were pooled and dialyzed against 10 mM EDTA and stored at 4 °C.

Preparation of Lipoproteins and ApoA-I-Lipoproteins were isoelectrofocusing (IEF) of plasma lipoproteins was carried out in an Elphor VAP22 apparatus calibrated in 20 mM phosphate, 0.5 mM NaCl, pH 7.4, 10 mM EDTA at 4 °C. Proteins were eluted with the same buffer at a flow rate of 0.2 ml/min at 4 °C. Fractions of 0.5 ml were collected. To detect clusterin, 100 μl aliquots were applied to nitrocellulose using a dot-blot microfiltration apparatus (Bio-Rad, Zürich, Switzerland), for apoA-I and apoB detection only 10 μl of the fractions were used. Specific antibodies to apoA and apoB (Calbiochem, La Jolla, CA) and asci fluid of murine monoclonal antibodies against clusterin were diluted 1:2,000 in incubation buffer. Immunostaining was performed as described for dot-blot assays below.

Dot-blot Assays—2.5 μg of purified delipidated apoA-I, ultracentrifugation-purified HDL (d=1.06-1.21 g/ml), LDL, and bovine serum albumin were spotted onto nitrocellulose. The filter was blocked with 0.1% BSA in incubation buffer containing 1% gelatine, 10 mM Tris-HCl, pH 8.4, 150 mM NaCl, 5 mM EDTA. The filters were incubated with clusterin at a concentration of 2.5 μg/ml in incubation buffer for 1 h at room temperature. Binding of clusterin was detected with monoclonal antibodies II-F4 or CLI-9 and peroxidase-labeled antimouse IgG using hydrogen peroxide and 4-chloronaphthol as the chromogenic agents.

Analytical Isotachophoresis of Lipoproteins—Normal EDTA-plasma, HDL-deficient EDTA-plasma from two different Tangier patients (patients I and II in Ref. 29), and purified apoA-I-clusterin complexes were studied by analytical isotachophoresis as described (30-32). The isotachophoretic mobility of purified apoA-I-clusterin complexes was compared with that after addition of purified apoA-I-clusterin to HDL-deficient plasma of the patients suffering from Tangier’s disease (29). Absorption at 570 and 254 nm was recorded on line during separation.

Preparative Isotachophoresis of Lipoproteins—Preparative separations of plasma lipoproteins was carried out in an Elphor VAP22 apparatus (Bender & Hobein, Munich, Federal Republic of Germany) apparatus (31, 32). Fresh fasting EDTA-plasma, HDL-deficient plasma samples from the Tangier patients, and HDL samples purified by ultracentrifugation were adjusted to a final protein concentration of 1 mg/ml in terminating buffer containing sugars (32). Equal amount of total proteins from the collected fractions were analyzed by SDS-PAGE on 12% polyacrylamide gels. After electrotransfer onto nitrocellulose membranes, clusterin was detected by immunostaining as described (1).

RESULTS

Affinity purification of apoA-I-clusterin complexes using the immobilized monoclonal antibody II-F4 (9) is shown in Fig. 1 (left panel). In all attempts made to remove unspecifically bound proteins with detergent-free washing buffers, a second bound protein of 28 kDa was recovered from the affinity column at pH 2.5. Therefore, a second monoclonal antibody prepared against purified clusterin (CLI-9) was used for the affinity purification. This antibody has a different binding specificity, since it binds to both nonreduced and reduced clusterin in immunoblots, in contrast to II-F4 which only recognizes nonreduced clusterin (1). However, co-purification of clusterin and the 28-kDa protein was still observed suggesting a specific association between clusterin and the 28-kDa protein. Densitometric scanning of Coomassie Blue-
Fig. 1. Isolation of apoA-I-clusterin complexes from human plasma. Left panel, co-purification of a 28-kDa plasma component with human clusterin. Approximately 10 μg of purified proteins isolated from plasma by immunoaffinity chromatography on anti-clusterin-Sepharose was analyzed by SDS-PAGE under nonreducing conditions and the gel bands were visualized by staining with Coomassie Blue. Molecular weight markers are shown on the left. Middle and right panel, dissociation and elution of apoA-I from clusterin bound to anti-clusterin Sepharose with 0.5% Triton X-100 in PBS (lane 1) followed by elution of clusterin from the monoclonal antibody Sepharose with 0.2 M glycine, pH 2.5 (lane 2). Differentially eluted proteins were analyzed by SDS-PAGE and immunoblotting using a monospecific rabbit antibody against human apoA-I and apoA-II (middle panel, lanes 1 and 2) and a monoclonal mouse antibody against human clusterin (right panel, lanes 1 and 2).

stained gels showed that the amount of the isolated 28-kDa protein was about half of that for clusterin on a weight to weight basis.

To exclude that the 28-kDa protein is a specific fragment of clusterin and to determine its identity, the 28-kDa protein was separated from clusterin by SDS-PAGE under nonreducing conditions and blotted onto Immobilon for direct amino acid sequence analysis. The ten amino-terminal residues obtained are Asp-Glu-Pro-Pro-Gln-Ser-Pro-Trp-Esp-Arg and are identical to that reported for mature plasma apoA-I (33). Immunoblotting of apoA-I-clusterin complexes and HDL preparations separated by SDS-PAGE showed that the 28-kDa component indeed co-migrated with apoA-I from HDL and was also detected by antibodies to apoA-I at the same intensity (data not shown). These findings clearly indicate that the 28-kDa component represents intact apoA-I, which is a characteristic component of HDL.

We studied the nature of interaction between apoA-I and clusterin. By adding 0.5% Triton X-100 to the PBS washing buffer, we were able to completely dissociate the 28-kDa protein from clusterin. ApoA-I was quantitatively eluted in the detergent buffer (Fig. 1, middle panel, lane 1), whereas clusterin remained bound to the affinity column (Fig. 1, right panel, lane 1). The column-bound clusterin was then washed with PBS buffer and eluted with 0.2 M glycine, pH 2.5. Lanes 2 in Fig. 1 show that clusterin was not lost from the column during the washing steps and that it did not contain any apoA-I.

We therefore presumed that binding between apoA-I and clusterin was mediated by hydrophobic forces and that plasma lipids may be involved in the formation of HDL-like apoA-I-clusterin complexes. Electron micrographs of negatively stained apoA-I-clusterin preparations were consistent with this notion. The ultrastructural appearance of apoA-I-clusterin complexes are spherical particles the majority of which has considerable size uniformity. A small fraction of particles appears to represent dimers (e.g. see upper right corner of Fig. 2), multimers and aggregates and have a proportionally larger size. In addition, a fraction of smaller particles whose negative staining is weaker and sometimes irregular, are discernible on the micrograph of Fig. 2. The average diameter of typical well contrasted particles was measured as the distance between the negatively stained edges of individual particles. In Fig. 2 the mean values for individually dispersed, typical apoA-I-clusterin particles was 9.0 ± 1.0 nm. These values fall into the smallest size range of HDL particles, range III, 8.5–9.6 nm, as defined previously (34).

Since ultrastructural shape and size of purified apoA-I-clusterin complexes resembles that of HDL particles, we analyzed the amount and spectrum of lipids present in the apoA-I-clusterin preparations. The apoA-I-clusterin lipoprotein particle consists of 78% (w/w) protein and 22% (w/w) lipids. Cholesteryl ester, free cholesterol, triglycerides, and phospholipids in concentrated preparations were determined by quantitative TLC on a molar basis. The lipids were composed of 54% (mole/mole) total cholesterol, 42% phospholipids (phosphatidylcholine 85%, sphingomyelin 9%, other phospholipids 6%), and 4% triglycerides. The molar ratio between unesterified cholesterol and esterified cholesterol amounted to 0.58. Thus, the apoA-I-clusterin complex is a lipoprotein particle of unusual composition in that it has a very high protein content and is rich in free cholesterol.

The specific interaction between apoA-I free clusterin and apoA-I was tested by dot-blot assays with purified filter-bound proteins and lipoproteins in the presence and absence of detergents. 2.5 micrograms of purified apoA-I, HDL, LDL, or BSA was spotted onto nitrocellulose filter membranes, remaining sites of the filter membrane were blocked by gelatin-containing TBS. Clusterin binding was visualized by immunostaining using monoclonal antibodies to human clusterin. We found that apoA-I free clusterin at a concentration of 2.5 μg/ml bound to purified apoA-I and to a lesser extent also to ultracentrifugation purified HDL, but not to LDL or BSA (Fig. 3). This interaction was completely abolished when 0.04% Tween 20 was present in the incubation buffer. The results are consistent with the above observation that nonionic detergents dissociate clusterin from apoA-I-lipid complexes. Furthermore, the experiments suggest that clusterin specifically associates with apoA-I and apoA-I-containing
was adsorbed to nitrocellulose using a dot-blot microfiltration apparatus. After blocking with 3% ovalbumin in TBS, filters were incubated with 10 μg/ml clusterin from which apoA-I had been dissociated, in the absence (−) or presence (+) of 0.05% Tween 20. Bound clusterin was visualized by immunostaining using monoclonal rabbit anti-mouse IgG antibodies.

Clusterin was visualized by immunostaining using monoclonal anti-clusterin antibodies in combination with peroxidase-coupled rabbit anti-mouse IgG antibodies.

Rabbit antisera against clusterin in combination with peroxidase-coupled rabbit anti-mouse IgG antibodies.

Bound clusters were then washed with 20 volumes of PBS to remove any residual detergent. Fresh plasma diluted in PBS was then passed through the column and subsequently washed extensively with PBS. Bound proteins were eluted with detergent-containing PBS in the same way as before (lanes 7–9 from the left). After washing with PBS, clusterin (Clu) was desorbed from the column with 0.2 M glycine, pH 2.5 (lane Gly). Equal volumes of the eluted fractions were analyzed by SDS-PAGE under nonreducing conditions. Proteins on the 12% acrylamide gel were stained with Coomassie Blue.

HDL particles and that hydrophobic interactions are involved in the formation of apoA-I-clusterin complexes.

To obtain further evidence for a biologically important, specific interaction between clusterin and apoA-I-lipid complexes, total plasma was passed over an anti-clusterin antibody-Sepharose column. All binding sites for clusterin were saturated by using an excess of plasma. Clusterin alone was specifically immobilized onto the affinity column by washing the affinity matrix with 0.5% Triton X-100 in PBS and then with an excess of PBS (Fig. 4, lanes 1–5 from the left). Fresh EDTA-plasma was chromatographed through the column a second time. The bound plasma proteins were eluted with 0.5% Triton X-100 in PBS (Fig. 4, lanes TX1–TX3) and then with 0.2 M glycine, pH 2.5 (Fig. 4, lane Gly). Fig. 4 shows that apoA-I and traces of apoA-II were taken up by the immobilized clusterin from total human plasma specifically. Only small amounts of a few additional unidentified plasma proteins were detected. apoA-I was completely removed from clusterin in nonionic detergent buffers, whereas clusterin was retained on the column. These results were verified by electroimmunoblot analysis of the different chromatographic fractions using monospecific rabbit sera to apoA (apoA-I and apoA-II) and monoclonal antibodies to clusterin (data not shown).

The native molecular mass of apoA-I-clusterin complexes in total fasting plasma was determined by gel filtration chromatography on a Superose 6B column. Previously it was reported that serum clusterin eluted from a gel filtration column in at least three different peaks, corresponding to the void volume, to the molecular mass of a monomer (68 kDa) and to the molecular mass of a dimer (8). Similar data were found for purified Sertoli cell-derived rat clusterin (SGP-2), which showed a high tendency to form dimers and large molecular weight aggregates in particular after freezing and thawing (35). Our gel filtrations experiments with fresh human EDTA-plasma yielded only one peak with clusterin immunoreactivity which eluted before albumin and slightly after immunoglobulins. High molecular weight forms of clusterin were not detected. Clusterin immunoreactivity was restricted to fractions that also contained apoA-I antigens (Fig. 5).

To exclude the possibility that the clusterin-apoA-I complex isolated by affinity chromatography is an artificial product generated by the interaction of immobilized clusterin with plasma HDL particles, we have determined the electrophoretic mobility of affinity-purified apoA-I-clusterin and of native plasma clusterin in isotachophoresis (ITP). This technique permits one to fractionate total lipoproteins and HDL into a number of subpopulations according to the mobility of the particles in a very short period of time (10 min). Lipoproteins are prestained with the nonpolar dye Sudanblack B and are monitored at 580 nm after migration. The individual peaks...
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from total plasma have been correlated with the different classes of lipoproteins, HDL, LDL, VLDL, and IDL (31, 32).

The 10 fractions collected from preparative free flow ITP of total plasma were analyzed by SDS-PAGE followed by immunoblotting. Clusterin immunoreactivity was detected in those preparative fractions, which correspond to peaks 4-6 in analytical ITP (Fig. 6, panel A) (32). In analytical runs of apoA-I-clusterin preparations most of the purified apoA-I-clusterin complexes were detected in fractions 4-6 by measuring the absorption at 254 nm as well as by detecting Sudan Black-stained lipids at 570 nm (Fig. 6, panel B, peaks b and c). A small proportion of purified apoA-I-clusterin complexes (peak a in panel B) was found in fractions 1 and 2 and showed a higher electrophoretic mobility than clusterin-HDL of human plasma. Except for this subpopulation of apoA-I-clusterin complexes which may form during purification or storage, purified apoA-I-clusterin had the same electrophoretic mobility as native clusterin from plasma samples indicating that the lipid-apoA-I-clusterin complex is not an artifact of affinity purification. In both samples (Fig. 6, panels A and B) clusterin co-migrated with the slow migrating subfraction III of ultracentrifugally purified HDL (peaks 4-6 in analytical ITP). Since spacing of individual lipoprotein fractions in ITP depends on the complexity of the protein sample analyzed, we also studied the isotachophoretic behaviour of clusterin-HDL in Tangier plasma samples which have a very low content of HDL. By immunoblotting ITP-fractionated plasma samples from Tangier patients, the level and isotachophoretic mobility of endogenous clusterin was found to be about the same as in normal subjects (data not shown). Since the HDL peaks 1-6 in the ITP profile of Tangier plasma lipoproteins are very low, we were able to monitor the migration of affinity-purified apoA-I-clusterin complexes (Fig. 6, peaks a-c in panel B) in analytical free flow ITP after adding them to total plasma of Tangier patients. Panel D of Fig. 6 shows that the majority of purified clusterin-HDL (panel D, peaks b and c) has the same isotachophoretic mobility as native clusterin-HDL in total human plasma (Fig. 6, panel A).

DISCUSSION

High density lipoproteins form a highly heterogeneous class of lipoprotein particles that interacts with a wide variety of cells and mediates various still poorly defined physiological functions. HDL levels are correlated with a low risk of premature atherosclerosis and are believed to improve the efficacy of reverse cholesterol transport. The relevant HDL subpopulations and the cellular processes underlying this antiatherogenic protective effect of HDL have not been identified (36-38). By using anti-clusterin immunoaffinity chromatography we have isolated a distinct protein-rich lipoprotein particle which contains apoA-I, clusterin, and only 22% lipids. As the clusterin concentration in normal human plasma is about 20 times lower than that of apoA-I, apoA-I-clusterin complexes represent about 2-4% of the total apoA-I protein in plasma assuming a 1:1 or a 2:1 molar ratio. Due to its low lipid content and resulting high hydrated density relative to globular plasma proteins, the apoA-I-clusterin particle does not float with HDLs or HDLs lipoprotein species obtained by sequential density gradient ultracentrifugation. Previous reports that purified plasma clusterin exists in monomeric, dimeric, and multimeric forms (8) may be due to losses of lipids and apoA-I, partial denaturation or aggregations of apoA-I-clusterin complexes during purification. Whether uncomplexed free forms of clusterin or multimers of it are actually present in the circulation at low levels remains to be explored. The high degree of particle size homogeneity as judged by electron microscopy and gel filtration chromatography strongly supports the view that apoA-I-clusterin complexes are the predominant form of clusterin in human blood plasma. However, the molecular forms of human clusterin that are found in the brain and in the tubular lumen of the testes may be different, since these body compartments are separated from the plasma compartment by tight physiological barriers.

The biochemical properties of clusterin are typical of apolipoproteins. Lipid-free preparations of clusterin and highly concentrated preparations of apoA-I-clusterin particles (not shown) show a strong tendency to aggregate, especially after freezing (35) and are poorly water soluble. The predicted α-helix content of clusterin is 41%. There are several helical segments of amphiphilic nature (residue 150-170, 215-240,
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300-350, and 406-420 in addition to an extensive amphiphilic \( \alpha \)-helical region at the amino terminus (residue 1-76) (1) which is conserved across the human, rat, and quail species. Similar amphiphilic structures and a high \( \alpha \)-helical content are shared by all apolipoproteins, explaining the relatively high similarity scores between apoA-I and clusterin noted previously (13). Therefore, clusterin may be regarded as a new specific apolipoprotein marker of a minor HDL subpopulation.

While this manuscript was submitted for publication, de Silva and colleagues (39, 40) reported on similar findings. These authors introduced the name apoJ for clusterin. Although most of their data is in accord with those published here, there are some differences which need further consideration.

Our data on the lipid content of apoA-I-clusterin particles (22 versus 11%) differ significantly from those reported by de Silva and co-workers (39, 40). In contrast to de Silva, we did not rely on commercially available enzymatic kits to determine individual lipid classes. In our hands these kits yielded much lower values for the lipid content than the quantitative TLC procedure developed by Schmitz and co-workers in 1984 (26). The fact that the majority of apoA-I-clusterin complexes were reported to have a density in the range between 1.18 and 1.23 g/ml (de Silva’s data, using sequential density gradient ultracentrifugation) is better supported by the higher lipid content of HDL-clusterin.

Comparing the micrograph of de Silva et al. (39, 40) with ours, the degree of microheterogeneity is apparently larger in the preparations of de Silva than in our clusterin-apoA-I samples. The authors report a total size range of 5-16 nm, whereas we find 9 nm for the diameter of a typical well dispersed and well contrasted spherical particle. The broader size range of the particles in de Silva’s report presumably results from a larger proportion of denatured or aggregated material that they subjected to alkaline elution conditions during immunoaffinity purification. In our hands, stability of HDL-clusterin particles were found to be better after eluting them at pH 2.5-2.8.

In an attempt to define the protein heterogeneity of apoA-I- and apoA-II-containing HDL particles, several minor protein moieties that were not related to apoA-I, apoA-II, apoC-I, apoC-II, and apoE have been encountered by anti-apoA-I immunoaffinity chromatography (41). These putative apolipoproteins were called NA1 to NA6 and co-purified in small amounts with apoA-I-particles. NA1 to NA6 were hardly detectable in HDL purified by ultracentrifugation. On the basis of these data alone it was not clear whether the copurifying NA1 to NA6 proteins were taken up from the plasma by immobilized HDL particles unspecifically or whether NA1 to NA6 were characteristic markers for genuine minor HDL subpopulations. The amino-terminal sequence information now available for NA1 and NA2 (42) shows that NA1 and NA2 are the two disulfide-linked subunits of clusterin. Thus, the presence of small amounts of clusterin in anti-apoA-I affinity-purified HDL particles indicates that apoA-I-clusterin particles can be obtained from plasma by anti-apoA-I affinity chromatography as well as by anti-clusterin affinity chromatography as shown in this study.

In order to circumvent the perturbing effects of ultracentrifugation on HDL and HDL subpopulations, and to demonstrate that the purified apoA-I-clusterin particles represent genuine lipoproteins of native plasma, we have fractionated total plasma and purified apoA-I-clusterin lipoproteins by free-flow isoelectric focusing (31, 32). Most of the purified apoA-I-clusterin lipoproteins co-migrated predominantly with the slowest fraction of HDL (peaks 4-6) in total human plasma (32) and had the same isoelectric focusing mobility as the native apoA-I-clusterin complex in a plasma sample. This finding provides further evidence that the affinity-isolated apoA-I-clusterin particle truly represents a natural lipoprotein entity in whole human plasma.

The biological properties of the different ITP fractions containing HDL have been studied recently (32). The fast and slow migrating fractions of HDL are good promoters of the cholesterol efflux from cultured cells and exhibit a high nonspecific interaction with cell surfaces, whereas the intermediate fraction lacks this activity. Since HDL subfractions isolated by ultracentrifugation have been used, the functional role of apoA-I-clusterin particles in the interaction of HDL with macrophages was disregarded. By contrast, the slow migrating HDL fraction of total plasma lipoproteins encompass apoA-I-clusterin particles (Fig. 6, panel A). The latter may represent a biologically relevant proportion of HDL subspecies which could be involved in HDL metabolism and lipid exchange between cells and lipoproteins of the plasma and interstitial fluid (37, 38).

The capability of purified clusterin and apoA-I-clusterin lipoproteins to interact with cell surfaces is supported by the following observations. Purified clusterin of seminal plasma and apoA-I-clusterin lipoproteins induce aggregation of many different types of dispersed cells from different species in vitro (43). This process does not appear to depend on a specific cell surface receptor. In vivo, clusterin also shows an affinity for cell membranes especially of those cells, which are damaged, abnormal, or dying (2, 44). For example, clusterin was detected on the cell surface of spermatozoa which were predominantly morphologically abnormal (2). In necrotic or involving tissues which express very high levels of clusterin, the protein is found in association with dying cells (19, 44, 45). Clusterin and apoA-I-clusterin complexes could thus assist in the mobilization, uptake, and redistribution of lipids from damaged or lipid loaded cells.

A role for clusterin in lipid transport and local lipid redistribution among cells in peripheral tissues is further consistent with the wide distribution of clusterin and with the strong inducibility of its expression in response to mechanical, chemical, or metabolic cell damage (19). The clusterin (SCP-2) message is most abundant in testis, epididymis, liver, and brain, low levels are detected in kidney, spleen, and the mammary gland (4, 12). Thus the tissue distribution of clusterin resembles that of apoA-I and apoE (46). In addition to this analogy, the apoA-I and apoE genes share the feature of inducibility with clusterin (16, 17, 19). ApoA-I and apoE are induced in nonneuronal bystander cells after a denervating crush injury of peripheral nerves distal to the lesion (47, 48). Increased levels of apoE and apoA-I lipoproteins were measured in the local environment of the regenerating nerve (48). A comparable upregulation of clusterin in the nervous system has recently been observed in scrapie-infected hamster brains and in the hippocampus of Alzheimer patients (14). Increased clusterin expression may indicate ongoing repair and remodelling processes of the diseased brain tissue and could be part of a lipid redistribution process similar to that described during nerve repair in the periphery.

In view of our results, the role of clusterin in the process of programmed cell death becomes much clearer. In contrast to the hypotheses proposed previously that clusterin may directly activate an apoptotic suicide mechanism, enhance the opsonization of cell debris, or function as a chemoattractant

\[ \text{B. Lowin, D. E. Jenne, and J. Tschopp, manuscript in preparation.} \]

\[ \text{D. E. Jenne and J. Tschopp, unpublished data.} \]
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(19), the proposed role for clusterin in lipid scavenging, lipid transport, and lipid recycling is in harmony with its sequence features, with its expression in normal tissues like Sertoli cells, ependidymal epithelial cells, and hepatocytes and with its unique capacity to bind plasma membrane lipids in conjunction with apoA-I. The capability of clusterin to neutralize the cytolytic potential of nascent terminal complement complexes appears to represent a second biologically important function (1, 49, 50) which may serve to minimize tissue damage in complement-mediated cytotoxic defense mechanisms.

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