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Heat Shock Protein 60 Is a High-Affinity High-Density Lipoprotein Binding Protein

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A new 55-kDa HDL/apolipoprotein binding protein was demonstrated in plasma membrane preparations of the human cell lines and primary cultured hepatocytes. Analysis of specific binding by ligand immunoblots of HDL, apoA-I, and apoA-II to a partially purified 55-kDa PA-I plasma membrane preparation demonstrated a $K_{d,HDL}$ = 50 nM (10 μg/ml), $K_{d,apoA\text{-}II}$ = 20 nM (0.4 μg/ml), and $K_{d,apoA\text{-}I}$ = 330 nM (10 μg/ml). Following preparative SDS–PAGE electrophoresis of a plasma membrane preparation isolated from human PA-I cells, fractions with apoA-II binding activity were collected, concentrated, and subjected to two-dimensional electrophoresis. Internal microprotein sequencing of the 55-kDa protein band revealed the binding protein as being heat shock protein 60 (hsp60). The hsp60 monoclonal antibody LK-1 blocked apoA-II binding to the 55-kDa HBP preparation. In summary, these results provide a potential mechanism to explain the known association between immunity developed against hsp60 and the development of atherosclerosis.

Key Words: **high-density lipoprotein-binding protein; heat shock protein 60.**

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Abbreviations used: heat shock protein 60, hsp60; high-density lipoprotein, HDL; high-density lipoprotein-binding protein, HBP; apolipoprotein A-I, apoA-I; apolipoprotein A-II, apoA-II; high-density lipoprotein-binding site, HBS; scavenger receptor B-I, SR-BI; high-density lipoprotein-binding protein 2, HB-2; 60-kDa chaperonin, cpn60.

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High-density lipoproteins (HDL) have been proposed as mediators of reverse cholesterol transport, the process whereby cholesterol is removed from peripheral tissues and delivered to the liver for subsequent excretion (1). It has been demonstrated that in part the efflux of the plasma membrane cholesterol to HDL is a passive diffusional process, and it does not require the interaction of HDL apolipoproteins with the cell surface (2, 3). In contrast, part of reverse cholesterol transport appears to be an energy dependent process and is mediated by ABCA1, an ATP-binding cassette transporter, which was recently shown to be defective in Tangier disease (4, 5).

A number of HDL-binding proteins, potentially involved in HDL apolipoprotein interactions with cell surface binding sites, have been reported (6). These include HBP (7), BP-2 (8) and GRP94 (9); however, their physiologic roles have not been clarified. In contrast, SR-BI, a HDL receptor interacting with multiple ligands, has been reported as the primary protein responsible for cholesterol ester delivery to steroid producing cells and the liver (10). Although some data show that it may facilitate cholesterol diffusion to acceptor particles *in vitro,* it is not involved in HDL-mediated cellular signaling or energy dependent cholesterol pumping to the plasma membrane (11–13). The ABCA-1 cassette transporter has been recently identified to be the energy dependent phospholipid/cholesterol transporter (4, 5). By apoA-I cross-linking experiments with ABCA-1 overexpressing cells, ABCA-1 has been recently demonstrated to bind apoA-I (14, 15). This indicates that when overexpressed, ABCA-1 may mediate cholesterol efflux to lipid free apolipoproteins by direct binding of the apolipoproteins to the cell surface. Overexpression of ABCA-1 has not appeared to affect HDL binding (14) demonstrating that a substantial amount of "molten" (globular like state with well-defined secondary structure folded in a compact

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globular shape) apoA-I conformation (16), is required for the apoA-I binding. ApoA-II and apoC-I, apolipoproteins which also exist in the molten form (17, 18) when lipid free demonstrate a similar ability to efflux intracellular cholesterol to the plasma membrane (19).

Recently, an autoimmune response to hsp60, the monomer of cpn60 complex responsible for productive molecular conformational folding, has been shown to be associated with the premature development of atherosclerosis (20). In a large epidemiological study, serum antibodies to mycobacterial hsp65, which strongly cross reacts with human hsp60, were found to be significantly increased in clinically healthy subjects with sonographically demonstrable carotid atherosclerosis (21, 22). Furthermore, immunization of rabbits with mycobacterial hsp65 induces atherosclerosis in normocholesterolemic rabbits (23) and accelerates lesion formation in hypercholesterolemic rabbits (24). Upon the development of atherosclerotic lesions, hsp60 was highly expressed in the macrophages, smooth muscle and endothelial cells (25, 26) in the areas colocalizing with HDL staining. An immune response to mycobacterial hsp65, which shares 50% homology with human hsp60, has been implicated with the premature development of atherosclerosis (24, 26–28).

In this report, we describe a new HDL-binding protein that has a high affinity for apoA-II and HDL and but a lower affinity for apoA-I, and was identified to be hsp60. A hsp60 monoclonal antibody to sequence 383–449, which is identical in the first 24 amino acids to mycobacterial hsp65 (sequence 356–393), blocked apoA-II binding to hsp60. These findings provide a potential link with the HDL apolipoprotein cell binding and the development of atherosclerosis due to anti-hsp60 immunity.

METHODS

HDL and apolipoproteins. Human HDL_3 ($1.125 < d < 1.216$) were isolated from plasma of healthy donors by two repetitive centrifugations by the method of Redgrave (29). The HDL3 were passed through a heparin–Sepharose column, and an apoE-free HDL fraction was collected. Purified apoA-I and apoA-II were obtained from Sigma.

Cell lines. PA-I, HepG2, HeLa, RAW 264, J774, Caco-2, COS cells and human fibroblast were grown until 50–60% confluence in EMEM medium containing 10% FCS, and 100 μg/ml of kanamycin. Subsequently, cells were washed to remove residual serum with PBS and further cultured in Williams' E medium, containing 2 μg/ml insulin, 10^{-7} M dexamethasone, 100 μg/ml kanamycin and 20 mM Hepes for a 3-day period. The medium was replaced daily, and the cells were subsequently used for plasma membrane preparations. Human and rat hepatocytes were isolated as reported before (30) and cultured as specified for cell lines.

Preparation of plasma membranes. All procedures were carried out on ice. Confluent monolayers of cell lines were washed 3 times with ice-cold 10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride, removed by scraping with a rubber policeman, and then homogenized in 10 mM Tris–HCl (pH 8.0), 300 mM sucrose, 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride using a Polytron homogenizer at 24,000 rpm for 20 s. The supernatant obtained by centrifugation at 10,000*g* for 10 min was then centrifuged at 100,000*g* for 60 min. The supernatant was then removed, the pellet resuspended and boiled in SDS–PAGE sample buffer and then analyzed in the ligand immunoblot assay.

Ligand immunoblot assay for HDL-binding proteins. Samples of plasma membrane proteins were separated by 7.5% SDS–polyacrylamide gel electrophoresis (SDS–PAGE), under nonreducing conditions (31) and electrophoretically transferred (15 V, 18 h, 8°C) onto nitrocellulose membranes (32). The nitrocellulose membranes/strips were incubated for 2 h with blocking buffer (10 mM [Tris] HCl (pH 7.4), 150 mM NaCl, 5% [wt/vol] lipid deficient (5% wt/wt) low lipid milk powder) at room temperature. Subsequently, the strips were incubated for 45 min with HDL, apoA-I or apoA-II at 4°C in TBS buffer containing BSA at a concentration of 10 mg/ml. The strips were then washed with 10 ml of ice-cold blocking buffer without milk followed by fixation with 4% paraformaldehyde in 100 mM phosphate buffer, pH 7.4 for 30 min at room temperature. The strips were washed 3 times with blocking buffer, and incubated with goat anti-human apoA-I or anti-apoA-II (Boehringer-Mannheim) in blocking buffer for 1 h at room temperature. After washing with blocking buffer (5 times for 5 min), rabbit anti-goat IgG antibodies conjugated with alkaline phosphatase preabsorbed with human plasma proteins (Sigma) were added as the second antibody.

Preparative protein electrophoresis. For partial purification of the HDL-binding proteins, plasma membranes were prepared from approximately 10^9 PA-I cells grown until confluence on 500-cm^2 petri dishes. Membrane preparations were delipidated with chloroform/methanol/water (1:4:4) and dissolved in 10 ml of SDS-sample buffer. Separation was performed with a Bio-Rad Model 491 utilizing a 7.5% SDS–PAGE gel in a 37 mm gel tube. Fractions in a volume of 2 ml were collected and analyzed by ligand blotting, using apoA-II as the ligand. Fractions with apoA-II binding activity were collected, concentrated and dialyzed against TBS, pH 7.4. Preparations were stored at −70°C.

Two-dimensional electrophoresis. Two-dimensional electrophoresis was performed according of the method of O'Farrell (33) by Kendrick Labs, Inc. (Madison, WI). Isoelectric focusing was carried out in a glass tube of inner diameter 2.0 mm using 2% pH 4–8 ampholines (Gallard Schlesinger, Long Island, NY) for 9600 V-h. Approximately 25 μl (100 μg) of partially purified HDL-binding protein were mixed with 25 μl of IEF sample buffer (Bio-Rad) and applied to the IEF gel. For the second dimension, a 7.5% SDS–PAGE gel was utilized. Proteins were electroblotted onto BA83 nitrocellulose and characterized by ligand blotting, utilizing apoA-II at the concentration of 1 μg/ml. Ligand blotting was performed as outlined above. In separate experiments, gels were stained with Coomassie Blue.

Internal protein microsequencing. Internal micro-protein sequencing was performed by Kendrick Labs, Inc. as reported. Briefly, the band of interest was identified in Coomassie G250-stained gel by comparing with apoA-II ligand immunoblots. Pieces of the gel were cut, washed with 500–1000 μl of 0.05 M Tris, pH 8.5/50% acetonitrile for 20 min with shaking. The supernatant was discarded, and the wash was repeated twice. The washed gel pieces were dried completely in a Speed-Vac. In-gel digestion was performed by incubation of the dried pieces in 50 μl digestion buffer (25 mM Tris, pH 8.5) containing 0.08 μg trypsin for 20 h at 32°C. When digestion was complete, the gel pieces were extracted three times with 100 μl 50% acetonitrile/0.1% TFA each followed by shaking for 30 min, and the supernatants were transferred to a Hewlett–Packard HPLC injection vial and dried in a Speed-Vac. After redissolving in 200 μl of 0.1% TFA, sample was applied on HPLC utilizing Vydac C18 column. The elusion was performed at a flow of 0.2 ml/min with 1.5–65% gradient acetonitrile. Selected fractions were analyzed on a Perkin–Elmer Model 494 sequencer.

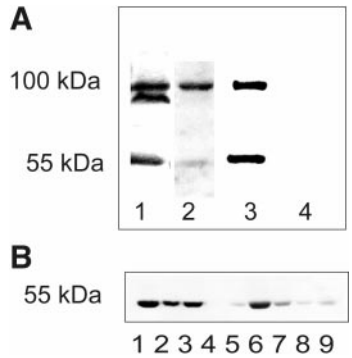


FIG. 1. Ligand blotting of plasma membrane preparations with HDL, apoA-I, and apoA-II. (A) Ligand blotting PA-I with: 1, HDL (10 μg/ml); 2, apoA-I (1 μg/ml); 3, apoA-II (1 μg/ml); 4, ligand-free control. (B) Ligand blotting of apoA-II (1 μg/ml) with plasma membrane preparations from 1, human hepatocytes; 2, rat hepatocytes; 3, HepG2 cells; 4, human fibroblasts; 5, HeLa cells; 6, RAW 264 cells; 7, J774 cells; 8, Caco-2 cells; 9, COS cells.

Western blotting. Partially purified the 55-kDa HBP or recombinant hsp60 were subjected to 7.5% SDS–PAGE followed by an electrotransfer to nitrocellulose membrane. After blocking in TBS (5% fat-free milk, pH 7.4), nitrocellulose membranes were incubated for 2 h at room temperature with LK-1 mouse monoclonal anti-human hsp60 (1:500; Sigma) antibodies in blocking buffer. After five washes for 3 min with blocking buffer, alkaline phosphatase-conjugated anti-mouse, -rabbit or -rat antibodies (all from Sigma) in blocking buffer at a dilution of 1:10,000 were incubated for 1 h at room temperature as second antibodies. Visualization was performed utilizing nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate in 5 mM $MgCl_2$, 100 mM Tris, pH 9.5.

RESULTS

Ligand Immunoblotting of HDL, ApoA-I and ApoA-II in PA-I Cell Line

Using a crude membrane preparation from PA-I cells, at least three different binding proteins could be observed by ligand blotting (Fig. 1A). When compared to HDL and apoA-I, apoA-II binds to the 55-kDa band with the greatest intensity (Fig. 1A, lanes 1–3). No detectable binding was demonstrated when incubating in ligand free solution, followed by the incubation with a mixture of anti-apoA-I and apoA-II antibodies (Fig. 1A, lane 4). A 55-kDa band was also observed in samples prepared from human and rat hepatocyte cells, RAW264, J774 macrophages, Cos, HeLa and Caco-2 cells. The 55-kDa band was barely detectable in human fibroblasts (Fig. 1B, lane 4).

Partial Purification of the 55-kDa Lipoprotein-Binding Protein

Using a partially purified fraction, the binding of apoA-II to the 55-kDa protein was further characterized. The 55-kDa band was partially purified by preparative gel electrophoresis. Collected fractions were concentrated and evaluated by apoA-II ligand blotting (Fig. 2). All fractions containing the 55 kDa apoA-II binding activity were concentrated and applied to a 5% SDS–PAGE column. Fractions collected after the second round of purification, were concentrated and extensively dialyzed against TBS, pH 7.4. This preparation was than used for two-dimensional electrophoresis and the further analysis of HDL, apoA-I and apoA-II binding.

HDL, ApoA-I, and ApoA-II Binding to a Partially Purified 55-kDa HDL-Binding Protein (HBP)

Several reports have demonstrated HDL-binding proteins, which bind both apoA-I and apoA-II apolipoproteins; however, lipoprotein-binding proteins with a higher affinity for apoA-II relative to apoA-I have not been previously reported (Oram J., 1992; Fidge, 1997; Kriger, 1996). To determine if the higher intensity of the 55-kDa band observed with apoA-II binding is the result of a higher affinity for this ligand, concentration-dependent curves were performed followed by Scatchard analysis. HDL, apoA-I and apoA-II all demonstrated concentration-dependent binding to the partially purified 55-kDa HBP by immunoligand blot analysis (Fig. 3). The highest affinity determined by Scatchard analysis was for apoA-II (K_d = 0.4 μg/ml), which corresponds to a K_d = 20 nM. Lower K_d's of 10 and 15 μg/ml were demonstrated for HDL and apoA-I, respectively, which when represented on a molar basis yielded a K_d = 50 nM for HDL (MW = 200 kDa) and a K_d = 500 nM for apoA-I (MW = 28 kDa).

Two-Dimensional Electrophoresis and Microsequencing of the 55-kDa HDL-Binding Protein

After resolution by 2D gel electrophoresis, the partially purified 55-kDa HBP primarily appeared as a triplet on both Coomassie stained gel (Fig. 4A) and by apoA-II immunoligand blot (Fig. 4B). The shape and position of the bands by either method were similar. The 55-kDa protein was cut out of the gel, treated with

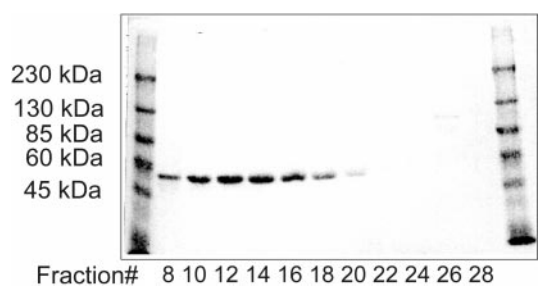


FIG. 2. Partial purification of the 55-kDa HBP utilizing preparative electrophoresis. 10 μl of each fraction was added with 2× SDS sample buffer and applied on 10% SDS–PAGE. After electrotransfer onto nitrocellulose, factions containing the 55-kDa HBP were revealed by ligand immunoblotting utilizing 1 μg/ml of apoA-II as a ligand.

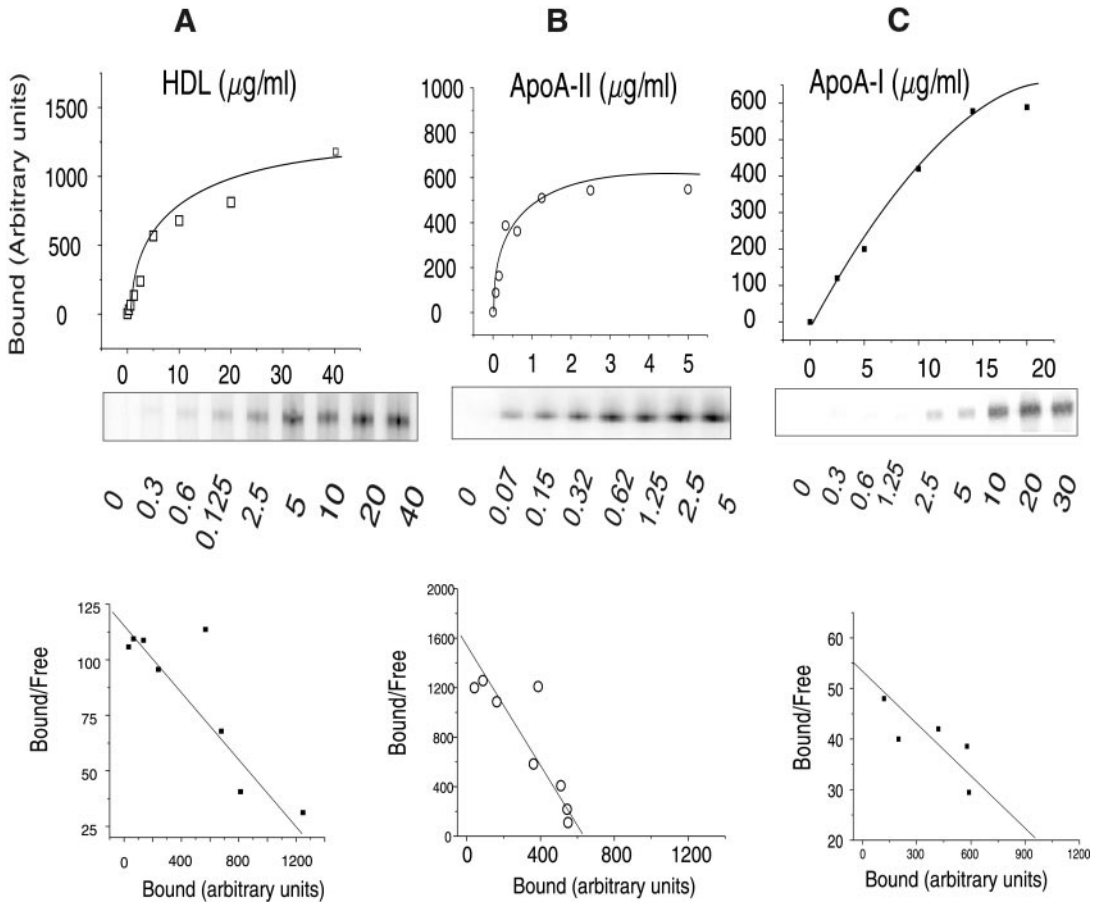


FIG. 3. Concentration-dependent binding of HDL, apoA-I, and apoA-II to the 55-kDa HBP preparation. Upper graphics: concentration-dependent binding of the ligand; middle graphics: immunoligand blotting with the ligands; lower graphics: Scatchard analysis of concentration-dependent binding of the ligands. In A, HDL; B, apoA-II; C, apoA-I were used as the ligands.

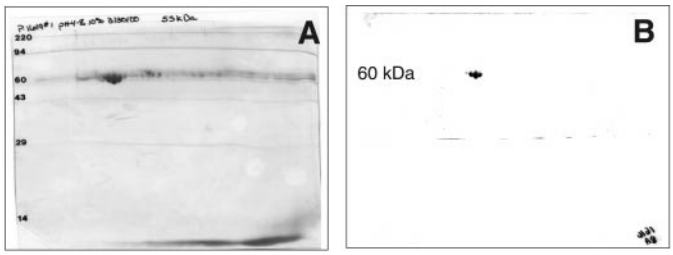
trypsin and subjected to HPLC. Six peptide peaks resolved by HPLC were used for protein microsequencing. The results of micro sequencing are shown in Fig. 4C. All sequences obtained fit the sequence of hsp60, a mitochondria matrix protein (34).

The Effect of Monoclonal Antibody (LK-1) to Hsp60 on ApoA-II Binding

When visualized by both apoA-II immunoligand blot and Western blotting with the LK-1 monoclonal antibody against hsp60, the 55 kDa HBP was located in a similar position (Figs. 5A and 5B). Similar results were obtained when recombinant human hsp60 (Sigma) was used for both immunoligand blotting and Western blotting (Figs. 5C and 5D). Furthermore, the LK-1 antibody blocked apoA-II binding to the 55 kDa HBP preparation in a concentration-dependent manner (Fig. 5E, lanes 2–7). No effect was observed when nonimmune control mouse antibody was coincubated with apoA-II (Fig. 5E, lane 1). The slightly higher molecular weight of recombinant human hsp60 is most likely attributable to the differences in post-translational modification between bacteria and animal cells.

DISCUSSION

ApoA-II is the second most abundant protein in HDL particles. It has been demonstrated that in contrast to apoA-I, human apoA-II overexpression in mice appears to be pro-atherogenic, by inducing fatty streak lesion formation in experimental animals (35, 36). In addition to its blocking effects upon a wide spectrum of enzymes of lipid metabolism (37, 38), apoA-II has also been recently demonstrated to convert HDL into a proinflammatory particle when overexpressed in mice (36). HDL particles isolated from apoA-II transgenic mice did not prevent oxidation of LDL and stimulated macrophage migration by inducing chemoattractant release in coculture. Although it was suggested that the presence of oxidized lipids or other factors in apoA-II transgenic HDL might promote the production of monocyte chemotactic protein-1, a direct interaction of



C

#	Peptide sequence	Position
1.	1 2 3 4 5 6 7 8 9 10 11 12 T V I I E Q S W G S P K	61-72
2.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 T L N D E L E I I E G M K F D	206-(221)
3.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 C E F Q D A Y V L L S E K K	237-250
4.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 V G E V I V T K D D A M L L (K)	345-359
5.	1 2 3 4 5 6 7 8 9 10 11 T L K I P A M T I A K	471-481
6.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 I M Q S S S E V G Y D A M A G	494-(516)

FIG. 4. Two-dimensional electrophoresis and microsequencing of the 55-kDa HBP preparation. (A) Coomassie staining of 2D gel; (B) ApoA-II immunoligand blotting; (C) internal microsequencing results of six peptide peaks separated by HPLC.

apoA-II enriched HDL with a HDL-binding protein was not ruled out as a potential mechanism.

A crude plasma membrane preparation from PA-I cells demonstrated the presence of the 55-kDa protein, which most significantly interacted with apoA-II and HDL. A much weaker binding was observed for apoA-I (Fig. 1A). Several cell lines were evaluated for the 55-kDa protein, including hepatocytes, fibroblasts and macrophages. Fibroblasts demonstrated apoA-II binding to the 55-kDa protein, only when larger amounts of membrane protein were used (data not shown). After two cycles of purifying the crude plasma membranes from PA-I cells by preparative 7.5% SDS–PAGE, the partially purified 55-kDa protein was utilized for HDL, apoA-I and apoA-II binding studies and Scatchard analysis. The highest affinity, with a K_d = 20 nM, was demonstrated for apoA-II. HDL and apoA-I were found to have K_d's of 50 nM and 500 nM respectively (Fig. 3). After two-dimensional electrophoresis, the band corresponding to the 55-kDa HBP by apoA-II ligand blotting was analyzed by micro-sequencing. All six HPLC peaks analyzed corresponded to the hsp60 sequence, a molecular chaperone with a molecular mass of 58 kDa (Fig. 4C).

Hsp60 is a highly conserved protein that is most abundant in mitochondria. Hsp60 forms an oligomeric cage-like structure with two rings of seven (bacterial) or eight (eukaryotic) subunits, with each ring surrounding a central cavity, which is known as cpn60. By repetitive cycles of folding and unfolding, cpn60 prevents irreversible aggregation of non-active conformations and keep proteins on the productive folding pathway (39). The oligomeric state of hsp60 during the ligand blot is not known, but it is possible that after the transfer to the nitrocellulose it could in part self-associate into the cpn60 form. A small amount of apparent hsp60 aggregates with molecular weights of approximately 240 kDa and higher were detected when performing non-reducing SDS by both anti-hsp60 Western blotting and apoA-II/HDL ligand blotting (data not shown), suggesting that oligomeric hsp60 can interact with HDL and apoA-II. Since the molecular weight limit for the peptide binding to cpn60 is approx-

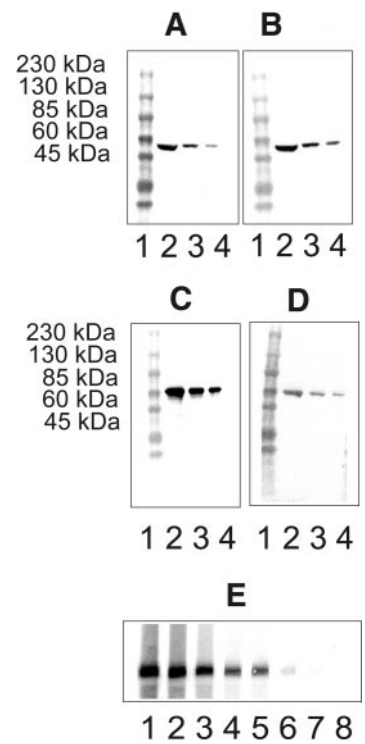


FIG. 5. The blocking effect of anti-hsp60 monoclonal antibody LK-I on apoA-II binding to the 55-kDa HDL-binding protein. (A) Western blotting of the 55-kDa HBP preparation with LK-1: lane 1, MW standard; lanes 2–4, various amounts of the 55-kDa HBP preparation (2, 4.5 μg; 3, 1.5 μg; 4, 0.5 μg). (B) Ligand blotting of 1 μg/ml of apoA-II to the 55-kDa HBP preparation; lane 1, MW standard; lanes 2–4, various amounts of the 55-kDa HBP preparation (2, 4.5 μg; 3, 1.5 μg; 4, 0.5 μg). (C) Western blotting of human hsp60 with LK-1: lane 1, MW standard; lanes 2–4, various amounts of hsp60 (2, 4.5 μg; 3, 1.5 μg; 4, 0.5 μg). (D) Ligand blotting of 1 μg/ml of apoA-II to hsp60: lane 1, MW standard; lanes 2–4, various amounts of hsp60 (2, 4.5 μg; 3, 1.5 μg; 4, 0.5 μg). (E) The effect of LK-1 on the binding of 1 μg/ml apoA-II with the 55-kDa HBP tested by ligand blotting: 1, nonimmune control (10 μg/ml); 2. LK-1 antibody-free control; 3. LK-1 (1 μg/ml); 4, LK-1 (2 μg/ml); 5, Lk-1 (5 μg/ml); 6, LK-1 (10 μg/ml); 7, LK-1 (15 μg/ml); 8, apoA-II-free control).

imately 70 kDa, both apoA-I and apoA-II could be easily accommodated. ApoA-I had a much lower affinity than apoA-II, perhaps because of apoA-I's structural features, including the higher α-helical content (80%) than apoA-II (30%) (16). Weaker folding and lower stability of lipid free apoA-II conformations may facilitate its avid interaction with hsp60 (18). Interestingly, HDL also showed high affinity binding to hsp60, which would not be expected given the typical size of most other ligands for cpn60/GroEL (40). The size of HDL (200 kDa) exceeds the cage size of the cpn60 complex (70 kDa). This suggests that hsp60 may bind HDL and perhaps apoA-I and apoA-II as a monomer or oligomer rather than in a highly structured cpn60 complex.

Although hsp60 predominantly resides in the mitochondria matrix, about 15–20% of hsp60 has also been reported in other cellular compartments with an unclear physiologic function (41, 42). A plasma membrane location has been reported for several cell types, including ovary cells (41), monocytic cell (43), endothelial cells (44) and lymphocytes (45). It has been reported that cell surface hsp60 is involved in L-system amino acid transport (45) and interacts with $p21^{ras}$ (46). Surface bound hsp60 has also been reported as a HIV virus gp41 binding protein which may be involved with anchoring the virus to the cell surface thereby facilitating contact between viral gp120 and its cellular receptors (47). In addition, the binding of amiloride (48) and misoribin (49) to hsp60 and its analogues is compatible with its role in molecular transport. These results suggest a cell surface role for hsp60 which would allow it to bind other extracellular ligands, such as HDL and apolipoproteins.

An immune response to hsp60 is associated with accelerated development of atherosclerotic lesions (28). Such observations have been demonstrated for diabetes, arthritis, system lupus erythematosus and others (27). Moreover, in animal experiments, immunization with mycobacterial hsp65, which shares a 52% homology with human hsp60, induced fat streak lesion formation (23, 24). An apparent 55-kDa molecular weight was observed for HBP with an anti-hsp60 monoclonal antibody LK-1 and ligand blotting with apoA-II. Recombinant human hsp60 demonstrated a slightly higher molecular weight by both apoA-II ligand blotting and LK-1 Western blotting when compared with the 55-kDa protein preparation from PA-I cells. The LK-1 antibody interacts with an epitope sequence located between 383 and 447 amino acids. The sequence between 383 AA and 417 AA is identical between human hsp60 and mycobacterial hsp65, which is hypothesized to be the antigen that induces autoimmunity against hsp60. When mixed with apoA-II, LK-1 blocked binding to the 55– kDa preparation in a dose dependent fashion (Fig. 5E). *In vivo,* an immune response to common sequences in human and mycobacterial hsp60 may interfere with the binding of apoA-II and HDL binding to hsp60 on the cell surface. Alternatively, the anti-hsp60 antibody may also mimic the effects of apoA-II when bound to hsp60.

If cpn60 is the form of hsp60 on the cell surface, the function of the cpn60 complex in the cell suggests two possible ways that it may be involved in HDL metabolism. The cpn60 complex has a high affinity for both lipid monolayers and bilayers (50). The C-terminal sequence is highly enriched in glycine and methionine, which has been demonstrated to be responsible for cpn60 insertion into membranes. Both the peptide binding activity and ATP hydrolysis of cpn60 were still present while within a membrane (50). Based on this model cpn60 has been proposed to roll in the lipid bilayer, resulting in, each peptide-binding site being cyclically exposed to intracellular and extracellular spaces. This model predicts that cpn60 complexes may function by transporting peptides, such as apolipoproteins into or out of cell. Apolipoproteins have been shown to undergo retroendocytosis, (51, 52), which may be important in the reverse cholesterol transport pathway. Alternatively, the repetitive cycles of folding and unfolding, cpn60 may affect equilibrium between the folded (highly helical) and molten (high affinity for lipids) conformations of lipid free apolipoproteins when located on the plasma membrane, which may affect their ability to dissociate from the cell and efflux lipid.

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