Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake

Shangzhe Xu,[1,*] Maria Laccotripe,[1,†] Xuewei Huang[2,*] Attilio Rigotti,[*] Vasillis I. Zannis,[†] and Monty Krieger[3,*]

Department of Biology,* Massachusetts Institute of Technology, Cambridge, MA 02139, and Section of Molecular Genetics, Cardiovascular Institute, Department of Medicine and Biochemistry,† Boston University Medical Center, Boston, MA 02118

Abstract The class B type I scavenger receptor, SR-BI, binds HDL, mediates selective uptake of HDL cholesteryl esters by cultured cells, and its expression is coordinately regulated with steroidogenesis in several endocrine tissues (adrenal, ovary, testes). SR-BI can also bind LDL and anionic phospholipids, which raised the possibility that HDL apolipoproteins might not participate directly in HDL binding. We have examined the ability of individual human HDL apolipoproteins (apoA-I, apoA-II, and apoC-III) reconstituted into phospholipid/unesterified cholesterol complexes to bind to murine SR-BI (mSR-BI) expressed in stably transfected cultured cells. All three apolipoprotein/phospholipid/unesterified cholesterol complexes specifically associated with mSR-BI expressing cells with high affinity and competed for the binding of HDL, while apolipoprotein-free complexes did not. Furthermore, lipid-free forms of these soluble apolipoproteins also competed for HDL and apolipoprotein/phospholipid/cholesterol complex association with mSR-BI, but locust high density lipophorin and bovine serum albumin were not effective competitors. Thus, all three of the HDL apolipoproteins (apoA-I, apoA-II, and apoC-III) tested can directly mediate binding to mSR-BI, and this multiligand apolipoprotein receptor may be responsible for at least some of the multilipoprotein and apolipoprotein binding activity previously observed in cells and tissues.—**Xu, S., M. Laccotripe, X. Huang, A. Rigotti, V. I. Zannis, and M. Krieger.** Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J. Lipid Res.* 1997. **38:** 1289–1298.

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Plasma high density lipoproteins (HDL) play critical roles in cholesterol metabolism and their plasma concentrations are inversely related to the risk for developing atherosclerotic disease (1). The mechanism of HDL's protective effect is not certain. In vitro experiments have suggested that HDLs may remove unesterified cholesterol from peripheral cells (2). The unesterified cholesterol in plasma HDL is converted to cholesteryl ester by the plasma enzyme lecithin: cholesteryl acyl transferase (LCAT) (3). In species that express plasma cholesteryl ester transfer protein (CETP), a significant fraction of HDL cholesteryl ester is transferred to other plasma lipoproteins for further metabolism (4). In rodents, and possibly in humans, there is an additional pathway for HDL lipid transport in which HDL directly delivers its cholesteryl esters to steroidogenic tissues for hormone synthesis (5) and to the liver for bile acid synthesis and secretion (6, 7). Both in vitro and in vivo studies have established that the mechanism of this direct delivery of HDL cholesterol to cells differs from that of the LDL receptor pathway, because it is not associated with endocytosis and degradation of the entire lipoprotein particle (8–11). These observations have suggested a novel, receptor-mediated, cellular mechanism for HDL cholesterol uptake called selective lipid uptake (8).

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; SR-BI, scavenger receptor class B type I; mSR-BI, murine SR-BI; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-III, apolipoprotein C-III; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; hCG, human chorionic gonadotropin; ACTH, adrenocorticotropic hormone; DPPC, 1,2-dipalmitoyl-L-3-phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-L-phosphatidylcholine; FAF-BSA, fatty acid-free bovine serum albumin; HDLp, high density lipophorin; PC, phosphatidylcholine; C, cholesterol; CHO, Chinese hamster ovary; ldlA, LDL receptor-deficient CHO cell line; ldlA[mSR-BI] cells, mSR-BI expressing ldlA cells; LBS, lipoprotein binding site.

[1]These authors contributed equally to this work.

[2]Present address: Duke University Medical School, Durham, NC 27705.

[3]To whom correspondence should be addressed.

Using either direct binding or ligand blotting assays, several laboratories have reported HDL binding activities in a variety of tissues from different species (reviewed in refs. 6, 7, 12), some of which may be involved in selective lipid uptake (13–15). In several cases, the HDL binding activities have exhibited a rather broad lipoprotein and apolipoprotein specificity. For example, these HDL binding sites can interact with intact lipoproteins, including HDL itself, VLDL, LDL and IDL, as well as isolated apolipoproteins, such as apoA-I, apoA-II, apoC- III, and apoE (13–26). These observations are intriguing not only because they suggest that some of the binding sites may represent multiligand lipoprotein receptors (27), but also because there are multiple HDL species containing varying combinations of apolipoproteins (6, 7, 24, 28, 29). The principal apolipoprotein components of HDL are apoA-I and apoA-II, and minor components can include apoA-IV, apoCs, apoD, and apoE (28). These apolipoproteins can have major structural roles or serve as regulators of HDL metabolism (7, 28). It is likely that some of the apolipoproteins are involved in the recognition of HDL by cell surface receptors. Indeed, recent studies of transgenic and knockout mice suggest that apoA-I and apoA-II may play fundamentally different roles in HDL and lipid metabolism (30–37). For example, there is a significant reduction in cholesteryl ester accumulation in steroidogenic tissues of apoA- I, but not apoA-II, deficient knockout mice (34), suggesting a special role for apoA-I in the delivery of HDL cholesterol to these tissues.

We have recently established that the class B scavenger receptor, SR-BI, is an HDL receptor that can mediate selective cholesteryl ester uptake from HDL in transfected cultured cells (38). Immunochemical analysis of SR-BI expression has established that, in rodents, SR-BI is expressed most abundantly in the liver and steroidogenic tissues (38, 39), sites where selective uptake of cholesterol from HDL is greatest. In addition, studies of the in vivo regulation of SR-BI protein expression by hormones, including estrogen, human chorionic gonadotropin (hCG), and ACTH, have shown coordinate regulation of SR-BI expression, selective uptake of HDL cholesterol, and steroidogenesis (39–41). These studies have suggested that SR-BI is a physiologically relevant receptor for the selective uptake of HDL cholesterol and that a detailed characterization of the mechanisms by which SR-BI binds HDL and mediates selective uptake will provide useful insights into HDL metabolism.

In the current study, we have explored the mechanism by which SR- BI recognizes HDL. Previous studies showed that SR-BI can bind to LDL as well as HDL (42), and that anionic, but not cationic or zwitterionic, phospholipid liposomes can bind to SR-BI with very high affinity (43). Because these lipoproteins do not share common apolipoprotein components (unlike HDL, LDL contains essentially only one apolipoprotein, apoB), these observations raised the possibility that the lipids on the surface of lipoproteins are the principal determinants of binding to SR-BI and that the apolipoprotein constituents do not participate in binding. To determine whether the apolipoprotein components might play a direct role in SR-BI binding, we examined the ability of individual human HDL apolipoproteins reconstituted into phospholipid/unesterified cholesterol complexes to bind to murine SR-BI (mSR-BI) expressed in stably transfected cultured cells. These complexes did not contain cholesteryl esters. Native HDL binding was also examined for comparison with these in vitro generated complexes. Unexpectedly, we found that apolipoproteins could mediate association with SR-BI and that all three of the HDL apolipoproteins tested, apoA-I, apoA-II, and apoC-III, could mediate the association of these phospholipid/unesterified cholesterol complexes with mSR-BI expressing cells and could compete for the binding of native HDL. Furthermore, lipid-free forms of these apolipoproteins could effectively compete for association with SR-BI. Others have previously reported multilipoprotein binding activities in tissues and cells with broad lipoprotein and apolipoprotein specificity (6, 7, 12–26). SR-BI may be responsible for some of these multilipoprotein binding activities.

EXPERIMENTAL PROCEDURES

Materials

Reagents (and sources) were: sodium [^{125}I]iodide and 1,2-dipalmitoyl-L-3-phosphatidyl[N-methyl-^{3}H]choline ([^{3}H]DPPC) (Amersham Corp., Arlington Heights, IL); fatty acid-free bovine serum albumin (FAF-BSA), cholesterol, sodium cholate, and 1-palmitoyl-2-oleoyl-L-phosphatidycholine (POPC) (Sigma Chemical Co., St. Louis, MO); dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA); Ham's F-12 medium, fetal bovine serum, and trypsin/EDTA (JRH Biosciences, Lenexa, KS); penicillin/streptomycin, glutamine, and G418 sulfate (GIBCO BRL Life Technologies Inc., Grand Island, NY). All other reagents were purchased from Sigma or other standard commercial sources as previously described (44).

Lipoprotein isolation, labeling, and characterization

Blood was obtained from healthy fasting human donors and HDL was prepared from pooled plasma (two donors for each preparation) by zonal centrifugation as

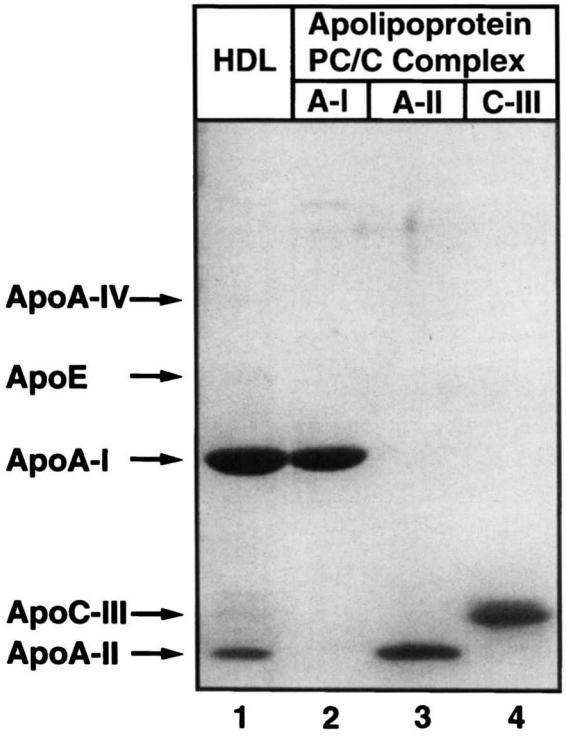

Fig. 1. SDS-polyacrylamide gradient gel electrophoretic analysis of HDL and apolipoprotein/PC/C complexes. Samples (10 μg protein/lane) of unlabeled HDL (lane 1), and apolipoprotein/PC/C complexes containing either apoA-I (lane 2), apoA-II (lane 3), or apoC-III (lane 4) were subjected to gel electrophoresis and Coomassie blue staining as described in Experimental Procedures.

previously described (45) and stored under nitrogen at 4°C. ^{125}I-labeled HDL (435–693 cpm/ng protein) was prepared using the iodine monochloride method (46). The protein contents of the HDL preparations, apolipoproteins and cells were determined by the method of Lowry et al. (47). Reducing SDS polyacrylamide gradient (6%–20%) gel electrophoresis followed by Coomassie brilliant blue staining was used to assess the apolipoprotein composition of the preparations. **Figure 1,** lane 1, shows that the major component in the HDL preparations was apoA-I (~28 kDa). The next most abundant component was apoA-II (~7 kD). We also observed a set of bands (~8–12 kDa) with lower staining intensities that represent apoCs and variable trace amounts of bands that presumably represent apoE (~34 kDa) and apoA-IV (~43 kDa). The identification of the minor apolipoprotein components was confirmed (not shown) by two-dimensional polyacrylamide electrophoresis (48). The relative amounts of the apolipoproteins varied somewhat from preparation to preparation. Native and radiolabeled HDL preparations were periodically monitored and preparations were discarded when evidence of abnormal electrophoretic mobility, probably due to oxidation, was observed. High density lipophorin (HDLp) isolated from the hemolymph of the gregarious locust, *Locusta migratoria,* was a generous gift from Dr. Nico P. Dantuma (Utrecht University, The Netherlands) (49). The principal apolipoproteins in this insect HDLp are apoLp-I and apoLp-II, which are derived from a common precursor that appears to be related to mammalian apoB (50).

Apolipoprotein/PC/C complex preparation

Purified apolipoprotein A-I (apoA-I), apolipoprotein A-II (apoA-II), and apolipoprotein C-III (apoC-III) were prepared as described previously (51, 52). Complexes comprising either phosphatidylcholine (PC) and cholesterol (C) alone (PC/C) or with apolipoproteins were prepared using the sodium cholate dialysis method (53) with minor modifications. The PC/C complexes were prepared using a weight ratio of 2.71:0.14 (PC:C). The apolipoprotein/PC/C complexes were prepared using a weight ratio of 1:2.71:0.14 (protein:PC:C). These compositions are the same as those previously reported by Jonas and colleagues (53–56). In brief, PC and cholesterol were mixed, dissolved in chloroform–methanol 2:1 and the solvent was evaporated under nitrogen. After drying, the lipids were suspended in 10 mM Tris-HCl, pH 8, 150 mM NaCl, 0.01% EDTA (buffer A) by vortexing and held on ice for 1 h. Then, sodium cholate (the final cholate/PC molar ratio was 1) was added and the mixture was incubated for 1 h on ice. Where indicated, individual apolipoproteins were added. The sodium cholate was then removed by extensive dialysis against buffer A at 4°C using tubing with a molecular weight cutoff of 12–14,000. Complexes were stored under nitrogen at 4°C. Radiolabeled complexes were prepared by adding [^{3}H]DPPC (125 μCi, 81 Ci/mol) to the unlabeled PC prior to the preparation of the complexes. The final specific activities ranged from 42 to 131 cpm/ng protein. The protein compositions of the preparations were confirmed using SDS polyacrylamide gel electrophoresis (e.g., see Fig. 1, lanes 2–4 representing apoA-I, apoA-II and apoC-III phospholipid/cholesterol complexes, respectively). The phospholipid concentrations of the preparations were determined using the method of Bartlett (57). The phospholipid to protein ratios (w/w) for the apolipoprotein/PC/C complexes were: apoA-I, 2.3–2.7; apoA-II, 2.7–3.1; and apoC-III, 1.4. The ratios of phospholipid to apolipoprotein for the apoA-I and apoA-II, but not the apoC-III, complexes were similar to that in the mixture used to prepare the complexes (see above). Apolipoprotein/lipid complex formation was verified by analysis with native polyacrylamide gradient (8–25%) gel electrophoresis (Pharmacia Phast gel system, Pharmacia Biotech, Piscataway, NJ). Our results were very similar to those previously described by Jonas,

Steinmetz, and Churgay (see Fig. 1, panel A, lane 1 of reference 54) for complexes prepared using the same procedure. The apolipoprotein/lipid complexes prepared for this study contained a mixture of particles exhibiting two discrete sizes. The majority of the particles (usually >50%, as much as 80%) exhibited a homogeneous electrophoretic mobility that corresponds to particles with a diameter of approximately 96–104 Å (not shown). The less abundant, larger forms had diameters of approximately 118–122 Å. ApoA-I/PC/C complexes with diameters of 96Å are expected to be discoidal, contain two apoA-I molecules per particle and have a structure similar to that of nascent HDL (55, 56). We presume that the numbers of protein molecules per particle in the apoA-II and apoC-III complexes were greater than 2 because their phospholipid/protein ratios were either similar to (apoA-II) or smaller than (apoC-III) that for the apoA-I complexes, while their protein molecular masses are significantly lower (~3.2-fold) than that of apoA-I. The apolipoprotein-free PC/C complexes are expected to be spherical unilamellar liposomes (58).

Cell culture

ldlA (clone 7) and ldlA[mSR-BI] cells were grown in culture as described previously (38, 42, 59). ldlA, clone 7, is an LDL receptor-deficient Chinese hamster ovary (CHO) cell mutant (60–62) that expresses very little SR-BI or HDL binding/selective uptake activity (38, 42). ldlA cells were maintained in monolayer culture with Ham's F12 medium containing 5% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (medium A). ldlA[mSR- BI] cells (38) are stabily transfected ldlA cells that express murine SR-BI (mSR-BI) and were maintained as stock cultures in medium A supplemented with 0.25 mg/ml G418 (medium B). All incubations with cells were performed at 37°C in a humidified 5% CO_2, 95% air incubator.

Cell association assays

SR-BI activity at 37°C was assessed by measuring cell association of radiolabeled ligands as previously described (38, 43, 44). In brief, on day 0 cells were plated at concentrations of 4.5–5 × 10^4 cells/well in 24-well dishes in medium A (ldlA) or medium B (ldlA[mSR-BI]). On day 2, the monolayers were washed with Ham's F-12 medium and then refed with 0.4 ml of medium C (Ham's F-12 containing 0.5% (w/v) FAF- BSA) with the indicated radiolabeled ligands (^{125}I-labeled HDL or apolipoprotein/[^{3}H]PC/C complexes) with or without unlabeled competitors. For direct association saturation curves (see Fig. 2, panels A–D), incubations were performed in the absence (total cell association, duplicate determinations) or presence (nonspecific cell association, single determinations) of a 40-fold protein mass excess of unlabeled HDL. For competition curves (see Fig. 2, panels E–I), duplicate incubations were performed in the absence or presence of the indicated unlabeled competitors. After a 1.5-h incubation at 37°C, the cells were washed twice with buffer B (50 mM Tris-HCl, pH 7.4 0.15 M NaCl) containing 2 mg/ml FAF-BSA, followed by one rapid wash with buffer B alone. The cells were then extracted with isopropanol (for ^{3}H-labeled apolipoprotein/lipid complexes) or solubilized with 0.1 N NaOH (for ^{125}I-labeled HDL), and radioactivity and protein determinations were made as previously described (38, 43, 59). The specific, high affinity cell association activities presented in the saturation curves represent the differences between the average total cell association and nonspecific cell association values and are expressed as nanograms of HDL, or apolipoprotein complex, cell-associated protein per mg cell protein. Cell association for the competition assays is presented as average values of percent of control in the absence of inhibitors. All findings were confirmed in at least two independent experiments.

To simplify comparisons between results with apolipoprotein-containing and apolipoprotein-free PC/C complexes, we expressed the amounts of apolipoprotein-free complexes as ng or μg of "protein equivalents". The protein equivalent values were calculated by determining both the phospholipid concentrations of the apolipoprotein-free and apoA-I-containing samples and the ratio of protein to phospholipid in the apoA-I complexes. The protein equivalent values were then assigned by defining the protein equivalent to phospholipid ratio of the apolipoprotein-free complex as the same as the measured protein to phospholipid ratio of the apoA-I-containing sample prepared at the same time. Thus, equal amounts of protein and protein equivalent represent equal amounts of phospholipids from the apolipoprotein-containing and apolipoprotein-free specimens.

RESULTS

To begin the analysis of the role that apolipoproteins play in the recognition of HDL by mSR-BI, we performed ligand association (**Fig. 2**), panels A–D) and competition (Fig. 2, panels E–H) assays using HDL and both apolipoprotein-containing and apolipoprotein-free phospholipid/cholesterol (PC/C) complexes. The purified human apolipoproteins incorporated individually into the PC/C complexes included apoA-I, apoA-II and apoC-III. Trace amounts of tritiated 1,2-dipalmitoyl-L-phospatidylcholine ([^{3}H]DPPC) were incorporated into the complexes to permit direct measurement

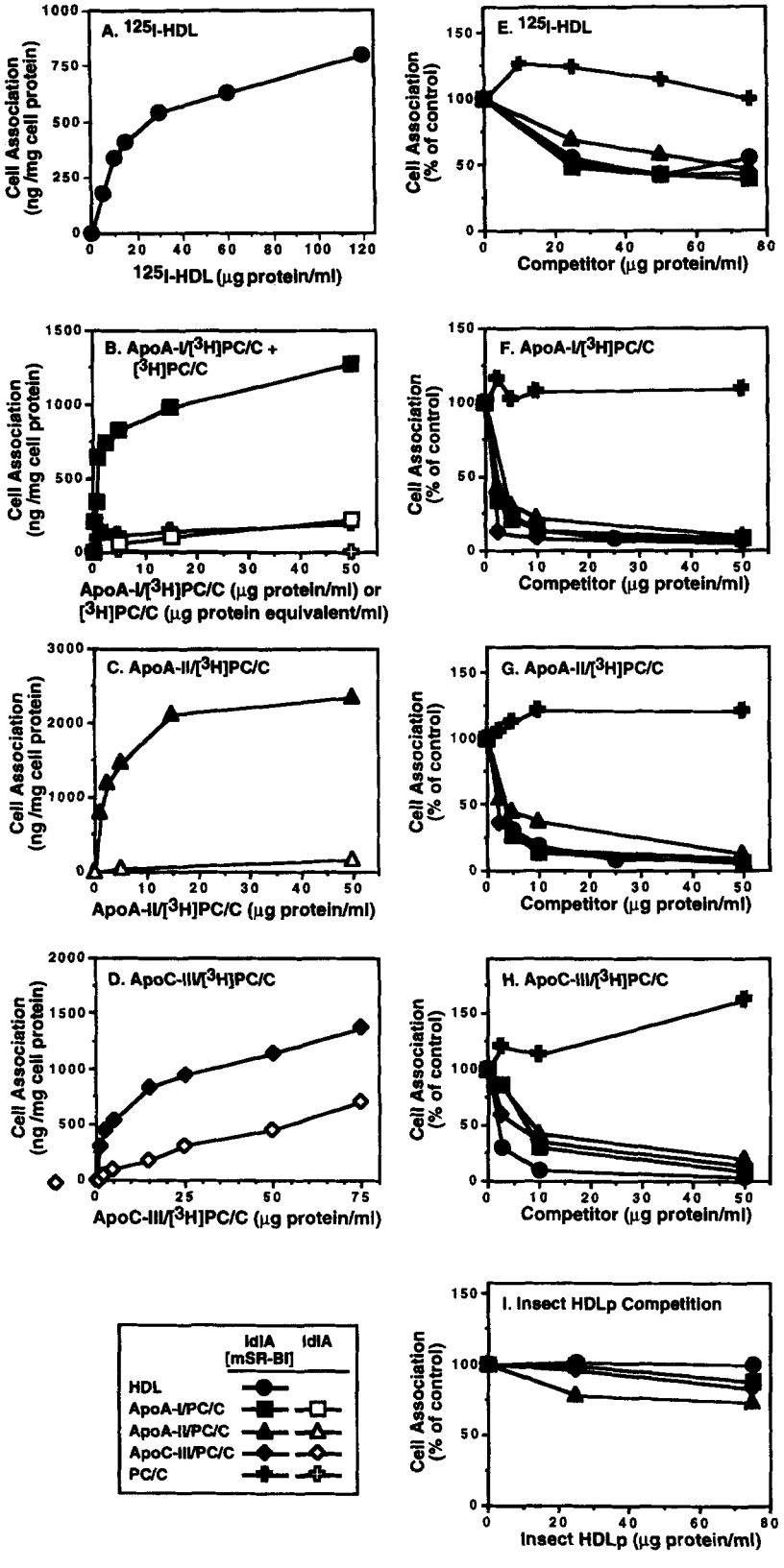

Fig. 2. Direct (A–D) and competition (E–I) assays of the association of HDL and apolipoprotein PC/C complexes with mSR-BI expressed in transfected cells. On day 2 after plating, mSR-BI expressing ldlA[mSR- BI] cells (solid symbols) or untransfected control ldlA cells (open symbols) were incubated for 1.5 h at 37°C with the indicated radiolabeled ligands and unlabeled competitors, and cell association was determined as described in Experimental Procedures. For the direct association saturation curves in panels A–D, specific (total − nonspecific) association values are shown, where nonspecific association was measured in the presence of a 40-fold (protein) excess of unlabeled HDL. The concentrations of labeled ligands (μg protein/ml) used in panels E–I were: ^{125}I-labeled HDL, 10; apoA-I/[^{3}H]PC/C, 0.5; apoA-II/[^{3}H]PC/C, 0.5; and apoC-III/[^{3}H]PC/C, 0.75. The 100% of control values (ng/mg cell protein) were: E, 607; F, 541 or 580; G, 1040 or 1118; H, 608 or 341; and for the four ligands in I, ^{125}I-labeled HDL, 201; apoA-I/[^{3}H]PC/C, 204; apoA-II/[^{3}H]PC/C, 393; and apoC-III/[^{3}H]PC/C, 275. The competition data with PC/C complexes without apolipoproteins (panels E–H, crosses) were determined in independent experiments from those shown with the other competitors. For the duplicate determinations with ldlA[mSR-BI] cells (panels A–D and the no competitor, 100% control data in panels E–I), the average of the differences between the duplicate determinations (v1 and v2), expressed as percent of the corresponding mean value [100*(v1 − v2)/0.5(v1+v2)], was 8.4% (median, 7.5%; standard deviation, 7%, n = 32). High density lipophorin (HDLp) from locust hemolymph was used in panel I.

of their association with the cells. The assays were performed at 37°C with transfected cells expressing mSR-BI (ldlA[mSR-BI], solid symbols) or with untransfected control cells (ldlA, open symbols). For several of the preparations, binding at 4°C was also measured and the results were similar to those observed at 37°C (not shown).

As we previously described, ^{125}I-labeled HDL exhibited high affinity association ($K_d \sim 20$ μg protein/ml) with cells expressing mSR-BI (Fig. 2, panel A), but virtually no association with untransfected controls (38, and data not shown). ApoA-I/[^{3}H]PC/C complexes exhibited saturable, high affinity association ($K_d \sim 1$ μg protein/ml) with ldlA[mSR-BI] cells, but virtually no binding to the untransfected control ldlA cells (Fig. 2B, squares). In contrast, when apolipoprotein-free PC/C complex association with the cells was measured (Fig. 2B, crosses), only very low, essentially background, levels were detected. This result is consistent with our previous report that SR-BI cannot effectively bind phosphatidylcholine/cholesterol (2:1) liposomes (43). Thus, incorporation of apoA-I into PC/C complexes conferred on these complexes the ability to bind to mSR-BI. ApoA-II/PC/C complexes (Fig. 2C, triangles) also associated with mSR-BI with high affinity ($K_d \sim 2$ μg protein/ml), as did apoC-III/PC/C complexes (Fig. 2D, diamonds, half-maximal association of ~3 μg protein/ml), although there was significantly higher background association with untransfected cells for apoC-III-containing complexes than for the other complexes or HDL. Maximal cell associations (ng of apolipoprotein per mg cell protein) were similar for the apoA-I and apoC-III complexes, while there was a higher (~60%) maximal association for the apoA-II complex. The molecular basis for this difference (stoichiometry of binding, conformation and number of apolipoproteins associated with the particles, etc.) has not been determined. Nevertheless, these data establish that all three major classes of apolipoproteins in HDL, apoA-I, apoA-II and apoC-III, can mediate binding to mSR-BI.

The ability of a strikingly diverse group of apolipoproteins to mediate interaction with mSR-BI might be due to the presence of multiple, independent binding sites or to a site(s) with intrinsically broad specificity. To begin to address this question, we examined the abilities of HDL and the apolipoprotein/PC/C complexes to compete with one another for cell association (Fig. 2 right, panels E–H). For these experiments, a fixed concentration of either ^{125}I-labeled HDL (10 μg protein/ml) or one of the apolipoprotein/[^{3}H]PC/C complexes (0.5–1 μg protein/ml) was added to mSR-BI expressing ldlA[mSR-BI] cells in the presence of the indicated concentrations of potential competitors. Cell association of the radiolabel was measured and expressed as % of control in the absence of competitor. For both ^{125}I-labeled HDL and all three of the apolipoprotein/[^{3}H]PC/C complexes, the apolipoprotein-free PC/C complex did not inhibit cell association (crosses). In contrast, HDL and all three of the apolipoprotein/PC/C complexes competed for labeled ligand association with similar, but not identical, apparent affinities. Because the concentration of ^{125}I-labeled HDL (panel E) was substantially higher than the concentrations of the apolipoprotein/[^{3}H]PC/C complexes (panels F–H), greater concentrations of the unlabeled competitors were required to inhibit ^{125}I-labeled HDL association (apparently larger K_is seen in panel E). These apparent differences in K_is were eliminated when the concentrations of the labeled ligands were comparable (not shown). The effective reciprocal ligand cross competition seen in Fig. 2 suggests that HDL and all of the apolipoprotein/PC/C complexes bind to the same, or perhaps overlapping, sites on mSR-BI.

In the experiments described above, purified human HDL apolipoproteins were incorporated into PC/C complexes both to generate HDL-like complexes and to facilitate labeling with [^{3}H]DPPC for direct association, rather than indirect competition, assays. We have also examined the ability of essentially lipid-free apolipoproteins to compete for radiolabeled ligand association with mSR-BI. **Table 1** shows that all three "free" HDL apolipoproteins could inhibit the association of ^{125}I-labeled HDL and of all three of the apolipoprotein/[^{3}H]PC/C complexes with SR-BI expressing cells. As was the case in Fig. 2, the higher concentration of ^{125}I-labeled HDL relative to that of apolipoprotein/[^{3}H]PC/C complexes resulted in lower relative amounts of inhibition at a fixed competitor concentration. Although it was possible that interpretation of these results might have been complicated if "free" apolipoproteins had been converted to PC containing complexes during incubation with the cells (e.g., see ref. 63), native gradient gel electrophoretic analysis of samples after incubation of free apolipoproteins with the cells under standard assay conditions showed no evidence for the formation of such complexes (data not shown). Thus, soluble "free" forms of the apolipoproteins as well as PC/C complexes with these HDL apolipoproteins can inhibit labeled ligand association, presumably due to direct competition for binding.

Our observation that all of the protein containing PC/C complexes as well as the free apolipoproteins competed with themselves and HDL for association with mSR-BI raised the concern that there might not be any specificity to this process, and that any protein might be able to effectively compete for HDL and HDL apolipoprotein association with mSR-BI. However, two findings establish that this is not the case. First, all of the assays shown in Fig. 2 were performed in the presence

TABLE 1. Lipid-free apolipoprotein inhibition of ^{125}I-labeled HDL and apolipoprotein/[^{3}H]PC/C complex association with mSR-BI expressed in ldlA[mSR-BI] cells

Competitor (50 μg protein/ml)	Cell Association (% of control)			
	^{125}I-HDL (10 μg protein/ml)	ApoA-I/[^{3}H]PC/C (0.5 μg protein/ml)	ApoA-II/[^{3}H]PC/C (1 μg protein/ml)	ApoC-III/[^{3}H]PC/C (1 μg protein/ml)
None	100	100	100	100
HDL	42	7	8	12
ApoA-I	64	15	19	12
ApoA-II	47	10	17	30
ApoC-III	44	12	17	30

ldlA[mSR-BI] cells were plated on day 0 and 2 days later cell association of ^{125}I-labeled HDL or one of the three radiolabeled apolipoprotein/PC/C complexes at the indicated concentrations was determined at 37°C in the absence or presence of the indicated competitors (50 μg protein/ml). The values represent the averages of duplicate determinations expressed as the percent of cell association in the absence of competitor. The 100% of control values (ng/mg cell protein) were: ^{125}I-labeled HDL, 236; apoA-I/[^{3}H]PC/C, 541; apoA-II/[^{3}H]PC/C, 391 or 1040; and apoC-III/[^{3}H]PC/C, 342–608. For the 23 sets of duplicate determinations represented here, the average of the differences between the duplicate determinations, expressed as percent of cell association in the absence of competitor, was 3.2% (median, 1.4%; standard deviation, 5%). Although each value shown represents observations from a single experiment, all of the results are from a total of six independent experiments.

of a large excess of bovine serum albumin (5 mg/ml). Thus, there was no high affinity binding of bovine serum albumin to the site(s) on mSR-BI that binds these ligands. Second, in competition experiments in which we added unlabeled insect high density lipophorin (HDLp, a lipoprotein) to the cells in presence of a fixed concentration of each of the four labeled ligands, there was little or no inhibition of cell association up to 75 μg protein/ml of HDLp (Fig. 2, panel I). In other experiments, concentrations of HDLp as high as 400 μg protein/ml did not inhibit ^{125}I-labeled HDL association (not shown). Thus, rather than representing a totally nonspecific binding process, the association of HDL and apoA-I, apoA-II and apoC-III with mSR-BI appears to be another example of broad, yet circumscribed, multiligand lipoprotein receptor activity (27).

DISCUSSION

In vitro and in vivo studies have strongly suggested that the class B scavenger receptor, SR-BI, is a physiologically relevant HDL receptor that can mediate selective cholesteryl ester uptake in the liver and steroidogenic tissues (38–41). In the current study, we have examined the ability of individual human HDL apolipoproteins reconstituted into phospholipid/cholesterol (PC/C) complexes to specifically associate with stably transfected cultured cells expressing mSR-BI. We found that apolipoprotein- free PC/C complexes could not bind to mSR-BI, but that apoA-I, apoA-II, or apoC-III incorporated into the complexes could mediate the high affinity and saturable association of these complexes with mSR-BI. Cross competition experiments suggested that native HDL and all three types of apolipoprotein-containing PC/C complexes bind to the same, or perhaps overlapping, sites on mSR-BI. Inhibition experiments also showed that lipid-free, soluble forms of apoA-I, apoA-II and apoC-III could effectively compete with both native HDL and the complexes, and thus, they presumably bound directly to mSR-BI. Not all proteins can bind tightly to the HDL binding site(s) on SR-BI, because neither bovine serum albumin nor insect HDLp inhibited association of HDL and the apolipoprotein complexes with the cells expressing mSR-BI.

In previous studies we established that native and modified LDL, HDL, and anionic phospholipids can bind with high affinity to SR-BI (38, 42, 43). Because these lipoproteins do not share common apolipoprotein components, these findings suggested the possibility that the lipids on the surface of lipoproteins were the principal determinants of binding to SR-BI and that the apolipoprotein constituents might not participate in binding. Based on the studies presented here, it seems likely that the apolipoprotein components (apoB, apoA-I, apoA-II and apoC-III, and perhaps other apolipoproteins not yet examined) with diverse primary sequences play important roles in mediating binding to SR-BI. The molecular basis of the broad apolipoprotein binding specificity of mSR-BI has not yet been established. Segrest and colleagues note in their comprehensive review (64) that one important feature common to all of the apolipoproteins is the presence of lipid binding amphipathic alpha helices. Perhaps one or more of these helices in these apolipoproteins provide common structural epitopes which are complementary to a binding surface(s) on SR-BI. Indeed, Mendez et al. (65) have reported that dimeric amphipathic helical peptides can compete for high affinity HDL binding sites on cholesterol-loaded fibro-

blasts. Future studies will be required to identify the binding determinants on both the apolipoproteins and SR-BI.

Prior to the identification of SR-BI as an HDL receptor with broad apolipoprotein binding specificity, other investigators had discovered and characterized broad binding specificity HDL receptor activities (6, 7, 12, 13–26, 65), some of whose activities are exceptionally similar to those of SR-BI. For example, a lipoprotein binding site (LBS), first reported by Bachorik et al. (16) and further characterized by Brissette et al.(13–15, 20, 21), is remarkably similar to SR-BI. LBS binds LDL, IDL, and HDL (13–16, 20, 21), as well as free and liposome-reconstituted apoA-I, apoA-II, apoC and apoE (13, 14, 21). As is the case with SR-BI, LBS mediates selective cholesteryl ester uptake from lipoproteins (13–15) and is expressed in the liver (16, 20, 21). Another HDL binding protein has been reported by Fidge and colleagues (18, 19). This protein exhibits broad lipoprotein and apolipoprotein specificity (although it apparently does not recognize apoC-III) (19), is expressed in the liver and adrenal gland (18, 66), and its expression in cultured adrenocortical cells is regulated by ACTH (66), all features in common with SR-BI. In addition, Hwang and Menon (26) showed that apoA-I, apoA-II, and apoC-III incorporated into dimyristoyl phosphatidylcholine vesicles bind to luteinized rat ovarian membranes with a specificity remarkably similar to that of SR-BI (ref. 38 and the current work) and that in vivo pretreatment of rats with human chorionic gonadotropin (hCG) increased binding. We have previously reported that administering hCG to male rats dramatically stimulates SR-BI expression in the Leydig cells of the testes (39). It seems possible that SR-BI may be responsible for some of these previously reported HDL binding activities. Additional studies will be required to establish the structural and functional relationships between the previously described HDL binding activities and SR-BI.

The functional relevance of the broad lipoprotein and apolipoprotein binding specificity of SR-BI is unknown. It is interesting to note that others have shown that selective cholesteryl ester uptake is not dependent on a single class of lipoprotein (8–11, 13–15, 67–74). HDL (8–11, 67, 68, 73), reconstituted HDL-like particles prepared with either apoA-I, apoCs, or native or modified apoE as the only apolipoproteins (70), IDL (13, 14), and LDL (15, 69, 71, 74) have all been shown to be sources of cholesterol for selective lipid uptake in vitro or in vivo. It seems likely that SR-BI may be responsible for some of this previously reported activity. It is important to recognize, however, that the mechanism of SR-BI-mediated selective lipid uptake has not been defined, and it is possible that only a subset of apolipoproteins that can bind to SR- BI can also mediate selective lipid uptake. Recent studies of cholesteryl ester accumulation in the steroidogenic tissues of apoA-I knockout mice (34) show that apoA-I plays a special role in this process, one which may influence selective lipid uptake. In addition, Barkia et al. (75) have observed substantial differences in the effects of apoA-I and apoA-II in HDLs on the efflux of cholesterol from cultured adipose cells. The identification of the broad apolipoprotein binding specificity of SR-BI can now serve as the basis for future detailed mechanistic analyses of multiligand lipoprotein receptor binding and selective lipid uptake.

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