Differential effects of HDL subpopulations on cellular ABCA1- and SR-BI-mediated cholesterol efflux

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Abstract Our objective was to evaluate the associations of individual apolipoprotein A-I (apoA-I)-containing HDL subpopulation levels with ABCA1- and scavenger receptor class B type I (SR-BI)-mediated cellular cholesterol efflux. HDL subpopulations were measured by nondenaturing two-dimensional gel electrophoresis from 105 male subjects selected with various levels of apoA-I in preβ-1, α-1, and α-3 HDL particles. ApoB-containing lipoprotein-depleted serum was incubated with [^{3}H]cholesterol-labeled cells to measure efflux. The difference in efflux between control and ABCA1-upregulated J774 macrophages was taken as a measure of ABCA1-mediated efflux. SR-BI-mediated efflux was determined using cholesterol-labeled Fu5AH hepatoma cells. Fractional efflux values obtained from these two cell systems were correlated with the levels of individual HDL subpopulations. A multivariate analysis showed that two HDL subspecies correlated significantly with ABCA1-mediated efflux: small, lipid-poor preβ-1 particles ($P = 0.0022$) and intermediate-sized α-2 particles ($P = 0.0477$). With regard to SR-BI-mediated efflux, multivariate analysis revealed significant correlations with α-2 ($P = 0.0004$), α-1 ($P = 0.0030$), preβ-1 ($P = 0.0056$), and α-3 ($P = 0.0127$) HDL particles. These data demonstrate that the small, lipid-poor preβ-1 HDL has the strongest association with ABCA1-mediated cholesterol even in the presence of all other HDL subpopulations. Cholesterol efflux via the SR-BI pathway is associated with several HDL subpopulations with different apolipoprotein composition, lipid content, and size.—Asztalos, B. F., M. de la Llera-Moya, G. E. Dallal, K. V. Horvath, E. J. Schaefer, and G. H. Rothblat. **Differential effects of HDL subpopulations on cellular ABCA1- and SR-BI-mediated cholesterol efflux.** ***J. Lipid Res.*** **2005.** 46: **2246–2253.**

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The inverse relationship between plasma levels of HDL, either cholesterol or apolipoprotein A-I (apoA-I) content, and coronary heart disease (CHD) has been demonstrated in many epidemiological, prospective, and intervention studies (1–6). The cardioprotective effect of HDL has been largely attributed to its role in reverse cholesterol transport (RCT), in which cholesterol that has been synthesized or deposited in peripheral tissues is returned to the liver for either recycling or excretion in the bile. This pathway involves several identifiable steps: *1*) ApoA-I is synthesized in the liver or small intestine. *2*) Phospholipids and cholesterol from cell membranes are transferred to apoA-I in the extracellular space in an ABCA1-dependent process that results in the formation of discoidal HDL (7–9). *3*) Free cholesterol (FC) is esterified by the action of LCAT, and the small, discoidal particles are converted to larger, spherical HDL (10). *4*) The interaction of spherical HDL with cholesteryl ester transfer protein transfers HDL cholesteryl esters (CEs) to apoB-containing lipoproteins (11). *5*) Cholesterol is transported to the liver, either via the selective uptake of HDL CEs by scavenger receptor class B type I (SR-BI) (direct RCT) or via the uptake of whole LDL by the LDL receptor in the liver (indirect RCT) (12).

Experiments with transgenic animals suggest that disturbances in one or more steps in RCT result in accelerated atherosclerosis, whereas the overexpression of key factors involved in RCT is atheroprotective (13). Moreover, humans who do secrete apoA-I and have defective ABCA1 have premature CHD (14, 15).

Some investigators believe that HDL has no significant role in RCT, because the amount of cholesterol that HDL carries back to the liver is insignificant compared with the amount carried by apoB-containing lipoproteins (16, 17). Concentrations of HDL even in the peripheral lymph are far higher than required for any step in RCT (18–21). Several investigators have suggested that HDL protects against atherosclerosis as a result of its anti-inflammatory and antioxidant functions as well as its actions in downreg-

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ulating adhesive molecules on the surface of vascular endothelium and inhibiting platelet aggregation (22, 23). Although these properties are well documented in vitro, these HDL functions have not yet been unequivocally established in vivo.

HDL is a heterogeneous lipoprotein class with different subspecies that vary in apolipoprotein and lipid composition, in size and charge, and in physiological functions (8, 24–26). We hypothesize that the diverse HDL subpopulations have different roles in RCT. Understanding the role of HDL in RCT is important, especially in light of epidemiological studies demonstrating significant correlations between specific HDL subspecies and the incidence of CHD (27–29). HDL_2 separated by ultracentrifugation contains mainly the α-1 HDL subpopulation, whereas HDL_3 contains all of the other apoA-I-containing HDL subpopulations (27).

Studies in recent years have identified cell surface receptors, ABCA1 and SR-BI, as significant factors in HDL metabolism (30–32). Consequently, the interaction between HDL and these cell surface proteins is under intense investigation. Early studies demonstrated that SR-BI could mediate the selective transfer of CE from HDL into cells and also promote the bidirectional flux of FC between HDL and the plasma membrane (33, 34). Despite intensive investigations, many aspects of the mechanism of SR-BI-mediated lipid flux (e.g., FC efflux and CE-selective uptake) between HDL and the plasma membrane remain unclear. With respect to the selective uptake of CE, it has been proposed that SR-BI functions in a two-step process in which the binding of HDL to the receptor is coupled to the flux of lipids (35–37). Studies using both synthetic reconstituted HDL (rHDL) and rat HDL showed that smaller HDLs promote more selective CE uptake, whereas other studies concluded that larger HDLs are able to deliver more CE compared with smaller HDLs (38, 39). HDL size affects SR-BI-mediated selective CE uptake, although the mechanism is poorly understood. Similar to CE selective uptake, SR-BI-mediated FC efflux is also size-dependent. Thus, large rHDL particles stimulated more cell cholesterol efflux than small particles when present at the same particle concentration (39). It has also been shown that differences in apoA-I conformation in different sized rHDL particles influence binding to SR-BI (39).

Almost all studies of cell cholesterol efflux as mediated by SR-BI or ABCA1 have used purified preparations of native lipoproteins or reconstituted particles; yet, in vivo, cells are exposed to a mixture of HDL particles. We exposed cells to the entire HDL fraction from individual serum specimens. These were prepared by the removal of apoB-containing lipoproteins using a polyethylene glycol (PEG) precipitation protocol, which does not dissociate apolipoproteins from mature HDL particles. Using this approach, we could establish the relative efflux efficiencies of each type of HDL particle by establishing the correlations between these subpopulations and ABCA1- and SR-BI-mediated cholesterol efflux. Our hypothesis was that different HDL subpopulations vary significantly in their ability to elicit cellular cholesterol efflux.

METHODS

Subjects

Plasma samples of 105 healthy male individuals (aged 18–65 years) were selected from a large pool of samples based on their specific HDL subpopulation levels and grouped accordingly: *1*) low preβ-1 (4.5 ± 1.1 mg/dl) and low α-1 (7.5 ± 1.7 mg/dl); *2*) low preβ-1 (4.1 ± 1.0 mg/dl) and high α-1 (32.6 ± 4.5 mg/dl); *3*) very low preβ-1 (2.3 ± 0.9 mg/dl); *4*) high preβ-1 (20.5 ± 2.2 mg/dl) and low α-1 (5.7 ± 1.0 mg/dl); *5*) high preβ-1 (19.9 ± 1.0 mg/dl) and high α-1 (32.7 ± 6.1); *6*) low α-3 (18.2 ± 2.1 mg/dl); and *7*) high α-3 (58.2 ± 1.3 mg/dl) (**Table 1**).

TABLE 1. Major characteristics of all subjects (n = 105) and subgroups of subjects (n = 15) selected with various preβ-1, α-1, and α-3 levels

Characteristic	All Subjects	Low Preβ-1, Low α-1	Low Preβ-1, High α-1	Very Low Preβ-1	High Preβ-1, Low α-1	High Preβ-1, High α-1	Low α-3	High α-3
Plasma lipid and apoA-I levels [mg/dl (SD)]								
LDL-cholesterol	115 (32)	124 (32)	94 (24)	97 (21)	131 (27)	128 (27)	106 (33)	125 (36)
HDL-cholesterol	47 (17)	32 (6)	58 (14)	43 (12)	36 (9)	68 (10)	47 (20)	46 (13)
Triglyceride	116 (78)	112 (39)	57 (15)	82 (27)	224 (92)	85 (32)	93 (52)	162 (99)
ApoA-I	126 (27)	101 (14)	132 (18)	115 (20)	120 (25)	160 (17)	114 (25)	138 (19)
ApoA-I levels in HDL subpopulations [mg/dl (SD)]								
Preβ-1	10.7 (8.0)	4.5 (1.1)	4.1 (1.2)	2.3 (0.6)	20.5 (1.8)	19.9 (1.1)	6.3 (3.2)	17.2 (6.2)
Preβ-2	2.2 (1.5)	1.3 (0.9)	3.1 (1.7)	2.2 (1.7)	1.6 (1.3)	2.8 (1.6)	2.9 (1.3)	1.9 (0.9)
α-1	20.0 (12.8)	7.5 (1.7)	32.6 (4.7)	22.3 (8.7)	5.7 (1.1)	32.7 (6.4)	28.3 (11.5)	10.8 (6.2)
α-2	39.2 (11.4)	34.1 (9.6)	41.9 (9.6)	37.4 (7.7)	33.4 (12.4)	52.0 (9.4)	36.5 (8.2)	38.9 (12.4)
α-3	36.6 (14.6)	40.3 (8.7)	26.2 (6.6)	30.2 (10.6)	48.7 (9.8)	34.4 (5.8)	18.2 (2.1)	58.2 (1.0)
Preα-1	6.3 (4.9)	2.6 (0.8)	10.9 (3.4)	8.3 (4.4)	1.7 (1.0)	7.8 (3.1)	10.1 (5.9)	2.6 (1.8)
Preα-2	6.6 (3.5)	5.1 (1.7)	9.3 (3.3)	8.6 (4.2)	3.5 (1.6)	7.2 (1.7)	8.1 (4.0)	4.2 (1.8)
Preα-3	4.0 (1.9)	5.3 (2.6)	3.6 (1.3)	4.3 (1.9)	4.3 (2.4)	2.9 (0.8)	3.1 (1.1)	4.4 (1.5)
Cholesterol efflux (4 h) mediated by (%)								
ABCA1	2.5 (1.5)	1.9 (1.0)	1.9 (1.1)	1.8 (1.1)	2.7 (1.6)	4.0 (1.3)	3.0 (1.8)	2.6 (1.5)
SR-BI	4.6 (1.3)	3.6 (0.8)	5.2 (1.2)	3.9 (0.6)	4.2 (1.0)	5.9 (1.4)	4.2 (1.1)	5.2 (1.3)

apoA-I, apolipoprotein A-I; SR-BI, scavenger receptor class B type I.

Serum lipid and apolipoprotein analyses

EDTA plasma was collected from all individuals after an overnight fast. Plasma was stored at −80°C until use. Total cholesterol, FC, HDL-cholesterol, and triglyceride (TG) concentrations were measured using standard automated enzymatic assays. LDL-cholesterol was calculated using the Friedewald formula. ApoA-I concentrations were measured with a turbidimetric immunoassay from Wako Diagnostics.

ApoA-I-containing HDL subpopulations were determined by nondenaturing two-dimensional gel electrophoresis, immunoblotting, and image analysis, as described previously (40). Briefly, 4 μl of plasma was applied and electrophoresed on a vertical-slab agarose gel (0.7%) in the first dimension at 250 V until the α-mobility front moved 3.5 cm from the origin. The agarose gel was sliced, and the strips were applied onto 3–35% nondenaturing concave gradient polyacrylamide gels. In the second dimension, gels were electrophoresed to completion at 250 V for 24 h at 10°C followed by electrotransfer to nitrocellulose membranes at 30 V for 24 h at 10°C. ApoA-I was immunolocalized on the membrane with monospecific goat anti-human primary and ^{125}I-labeled secondary antibodies [immunopurified rabbit F(ab′)$_2$ fraction against goat IgG]. The bound ^{125}I-labeled secondary antibody was quantified in a FluoroImager (Molecular Dynamics).

Quantitative image analysis of the apoA-I-containing HDL subpopulations

Preβ-mobility particles did not overlap with any other HDL particles, so they were easily delineated. Designation of the α-mobility HDL subpopulations was based on the integration of α-migrating HDL. Particles with faster than α-mobility in the same size ranges were designated as preα particles. Eleven apoA-I-containing HDL subpopulations were encircled (preβ-1$_{a,b}$, preβ-2$_{a,b,c}$, α$_{1–3}$, and preα$_{1–3}$), and signals were measured in each area and used to calculate the percentage distribution of HDL subpopulations. Sizes were determined from the molecular mass standard (^{125}I-labeled Pharmacia high molecular mass standards) run simultaneously on the same gel (**Fig. 1**). Results were expressed as averages of two parallel separations. ApoA-I concentrations of the subpopulations were calculated by multiplying percentiles by plasma total apoA-I concentrations. Because subjects were selected on the basis of total preβ-1 concentration, in the analyses we calculated with eight apoA-I-containing HDL subpopulations. However, in some analyses, we calculated with preβ-1$_a$ and preβ-1$_b$ separately.

Routine maintenance of cells and assay of cell cholesterol efflux

BSA, HEPES, and all reagents, solvents, and chemicals were obtained from Fisher Scientific or as specified in the text. PBS, MEM, and RPMI were purchased from Mediatech. FBS, bovine calf serum, enzymes, and antibiotics for cell culture were obtained from Sigma. Compound CP113,818 was a gift from Pfizer. Tissue culture flasks were obtained from Corning. All filtration products were obtained from Millipore. Other materials and reagents were obtained as noted.

Fu5AH cells were maintained in MEM supplemented with 5% bovine calf serum and antibiotics. J774 macrophages were maintained in RPMI supplemented with 10% FBS and antibiotics. For efflux experiments, 350,000 cells per well were plated on 24-well plates using 0.5 ml of the corresponding growth medium. After 24 h, the growth medium was replaced with 0.5 ml of labeling medium containing 2 μCi [^{3}H]cholesterol/ml and 2 μg/ml of the ACAT inhibitor CP113,818, and the cells were incubated in this medium for another day. Fu5AH cells were labeled with radioactive cholesterol in MEM containing 0.5% bovine calf serum. J774 cells were labeled using RPMI containing 1% FBS.

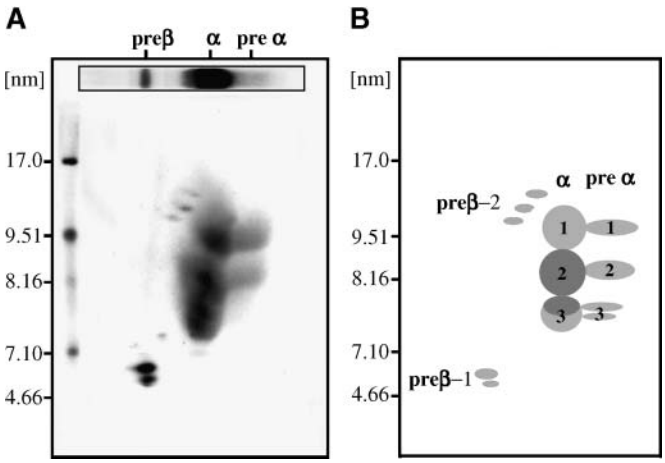

Fig. 1. The left panel shows the apolipoprotein A-I (apoA-I)-containing HDL subpopulations separated by two-dimensional nondenaturing agarose PAGE of a normolipidemic, healthy male subject. The rectangular inset represents the first-dimensional separation on an agarose gel (separation based on charge). A duplicate of that agarose strip was applied on the top of a concave gradient polyacrylamide gel (3–35%) and electrophoresed in the second dimension (separation based on size). Molecular mass standards are shown at left. The right panel shows a scheme of the apoA-I-containing HDL subpopulations in human plasma. The lighter shading indicates apoA-I-containing subpopulations; the darker shading represents apoA-I- and apoA-II-containing subpopulations.

Control HDL$_3$ (1.125–1.21 g/ml) was isolated by sequential centrifugation from human serum obtained from healthy, normolipidemic volunteers. Control human serum pools were also prepared from healthy normolipidemic volunteers. ApoA-I was obtained from human HDL. The apoprotein was isolated by anion-exchange chromatography using a fast-protein liquid chromatography method adapted from Weisweiler, Friedl, and Ungar (41). [1,2-^{3}H]cholesterol was purchased from New England Nuclear Life Science Products.

Just before the initiation of the efflux assays, the plasma was rapidly thawed and serum was prepared from the test plasmas as follows. Using a glass tube, 1 M $CaCl_2$ was diluted to a final concentration of 25 mM (1:40 dilution) in the plasma to be clotted, the mixture was incubated at room temperature for 1 h, and the plasma clot was removed by low-speed centrifugation to obtain the serum supernatants. Aliquots from these serum specimens were then treated with PEG solution to precipitate apoB-containing lipoproteins by adding 40 parts PEG solution (20% PEG in 200 mM glycine buffer, pH 7.4) to 100 parts serum. After a 20 min incubation, the precipitate was removed by high-speed centrifugation (10,000 rpm, 30 min, 4°C) to obtain the PEG supernatant containing the HDL lipoprotein fraction. SR-BI-mediated cell cholesterol efflux was measured as the fraction of radioactive cholesterol released from Fu5AH cells to 0.2 ml/well of MEM-HEPES containing either the test serum diluted to 2% or the same serum diluted to 2.8% after removal of apoB-containing lipoproteins as described below. ABCA1-mediated efflux was measured as the difference in release of radioactive cellular cholesterol to these same media from J774 control macrophages and J774 macrophages expressing ABCA1 (ABCA1 efflux = FC efflux from upregulated cells − FC efflux from control cells). To upregulate ABCA1 in J774 macrophages, half of the wells, plated with labeled J774 cells, were incubated for 16–18 h with 0.5 ml/well RPMI containing 0.2% BSA, 2 μg/ml CP113,818, and 0.3 mM cpt-cAMP. Labeled control J774 cells were also incubated for

TABLE 2. Univariate and multivariate regression analyses of ABCA1-mediated cholesterol efflux from J774 macrophages on apoA-I-containing HDL subpopulations

	Univariate Analysis				Multivariate Analysis			
Subpopulation	B	se(B)	t Value	*P* Value	B	se(B)	*t* Value	*P* Value
Preβ-1	0.0617	0.0174	3.54	0.0006	0.0686	0.0218	3.15	0.0022
Preβ-2	0.1039	0.0987	1.05	0.2953	0.0229	0.1145	0.20	0.8422
α-1	0.0157	0.0115	1.36	0.1758	−0.0131	0.0274	−0.48	0.6341
α-2	0.0359	0.0125	2.88	0.0048	0.0402	0.0200	2.01	0.0477
α-3	0.0068	0.0101	0.67	0.5050	−0.0188	0.0173	−1.08	0.2810
Preα-1	0.0185	0.0300	0.62	0.5384	0.0703	0.0736	0.95	0.3420
Preα-2	0.0044	0.0427	0.10	0.9190	−0.0818	0.0931	−0.88	0.3815
Preα-3	−0.0620	0.0777	−0.80	0.4265	0.0484	0.0947	0.51	0.6103

B, regression coefficient; se(B), standarad error (B); t, t-test [B/se(B)].

16–18 h with the same medium without cpt-cAMP. Before the addition of efflux medium containing the samples to be tested, all cell monolayers were gently washed once with 0.5 ml/well MEM supplemented with 1% BSA and once with 0.5 ml/well MEM. All efflux assays were performed in triplicate. The fraction of the total radiolabel incorporated into cell lipids released during incubation with the test samples was measured as follows. Radioactive cholesterol incorporated into cell lipids was extracted by incubating a set of triplicate wells of Fu5AH, control J774, and upregulated J774 cells not exposed to the test serum (T0 cells) overnight with 1 ml isopropanol/well. Total cellular cholesterol label was measured by liquid scintillation counting in aliquots of the isopropanol extracts after drying and resuspension in toluene. Radioactive cholesterol released to test samples after incubation with Fu5AH cells for 2 h or with control and upregulated J774 cells for 4 h was measured by liquid scintillation counting of 100 μl aliquots of efflux medium that had been filtered through 0.45 μm multiscreen filtration plates. All efflux values were corrected by subtracting the small amount of radioactive cholesterol released from triplicate wells of Fu5AH, control, and upregulated J774 cells incubated with serum-free medium.

To correct for interassay variability, control lipid-free human apoA-I and a pool of human serum were tested in parallel with the test samples in every assay. These internal controls were used to normalize the efflux values to control for the assay variation. Efflux from Fu5AH cells (SR-BI) was normalized on the basis of the efflux obtained with the human serum pool (2%). HDL_3 was used as a control for monitoring SR-BI-mediated efflux. ABCA1-mediated efflux from the J774 cells used the efflux obtained with lipid-free apoA-I (20 μg/ml) for normalization.

Data analysis

The data were examined graphically and numerically to determine whether transformations were needed before formal analysis. Simple and multiple regression models were fitted to assess each particle's contribution to efflux. Also, all 255 possible regression models, defined by all possible subsets of the eight particles, were fitted and their adjusted R^2 and Mallows' C_p statistics (42) were examined. Calculations were performed using the SAS package for Windows, version 8.2 (SAS Institute, Cary, NC), and SYSTAT, version 10.2.01 (Systat Software, Inc., Point Richmond, CA).

RESULTS

To evaluate the association of different HDL subpopulations with cellular cholesterol efflux, each serum sample was treated with PEG according to the protocol described in Methods to remove the apoB-containing lipoproteins that can contribute to efflux to varying degrees. Previous studies using a large number of specimens (>75) demonstrated that this procedure eliminated 97% of the apoB-containing lipoproteins while retaining >90% of apoA-I- and apoA-II-containing HDL particles (G. H. Rothblat, unpublished observations).

Major characteristics of all subjects (n = 105) and subgroups of subjects (n = 15) selected with various preβ-1, α-1, and α-3 levels are presented in Table 1. The mean apoA-I and HDL-cholesterol levels were highest in the group with high preβ-1 and high α-1 levels. The mean LDL-cholesterol and TG levels were highest in the high preβ-1/low α-1 group. HDL from subjects in the group with high preβ-1 and high α-1 promoted the highest level of ABCA1-mediated cholesterol efflux. HDL from subjects

TABLE 3. Univariate and multivariate regression analyses of SR-BI-mediated cholesterol efflux from Fu5AH cells on apoA-I-containing HDL subpopulations

	Univariate Analysis				Multivariate Analysis			
Subpopulation	B	se(B)	t Value	*P* Value	B	se(B)	*t* Value	*P* Value
Preβ-1	0.0545	0.0151	3.60	0.0005	0.0377	0.0133	2.83	0.0056
Preβ-2	0.2716	0.0822	3.31	0.0013	0.0392	0.0700	0.56	0.5772
α-1	0.0448	0.0091	4.95	<0.0001	0.0511	0.0168	3.05	0.0030
α-2	0.0744	0.0086	8.68	<0.0001	0.0452	0.0122	3.69	0.0004
α-3	0.0133	0.0088	1.52	0.1305	0.0256	0.0106	2.42	0.0174
Preα-1	0.0644	0.0254	2.54	0.0127	0.0121	0.0450	0.27	0.7890
Preα-2	0.0785	0.0363	2.16	0.0330	−0.0224	0.0569	−0.39	0.6951
Preα-3	0.0412	0.0677	−0.61	0.5444	0.0966	0.0579	1.67	0.0981

B, regression coefficient; se(B), standarad error (B); t, t-test [B/se(B)].

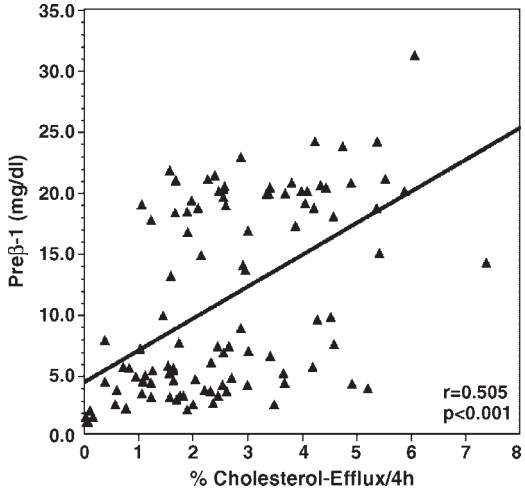

Fig. 2. Linear regression analysis of preβ-1 HDL subpopulation levels and ABCA1-mediated cholesterol efflux from J774 macrophage cells determined after 4 h of incubation.

in the groups with low preβ-1 (low preβ-1/low α-1, low preβ/high α-1, and very low preβ-1) promoted the three lowest levels of cholesterol efflux via the ABCA1 pathway. HDL from subjects selected with high α-1 (high α-1/high preβ-1, high α-1/low preβ-1) promoted the highest levels of cholesterol efflux via the SR-BI pathway.

In a univariate analysis, preβ-1 ($P = 0.0006$) and α-2 ($P = 0.0048$) were significantly related to ABCA1-mediated cholesterol efflux (**Table 2**). In multivariate analysis, the relationships between ABCA1-mediated efflux and preβ-1 ($P = 0.0022$) and α-2 ($P = 0.0477$) were significant. We ran analyses with all possible (255) combinations of HDL particles. All of the analyses suggested a role for preβ-1 and α-2 in ABCA1-mediated cholesterol efflux. The model that included all HDL particles as predictors of ABCA1-mediated efflux had an adjusted R^2 of 0.1194 ($P = 0.0086$). The regression model containing preβ-1 alone had an adjusted R^2 of 0.1003. Moreover, every model that included preβ-1 had an adjusted R^2 of 0.0924 or greater, whereas every model that excluded preβ-1 had an adjusted $R^2 < 0.0691$. The model containing preβ-1 and α-2 had an adjusted R^2 of 0.1440. When preβ-1_a and preβ-1_b were calculated separately, only the larger preβ-1_a correlated significantly with the cholesterol efflux via the ABCA1 pathway (data not shown).

SR-BI-mediated cholesterol efflux was handled in a similar manner. In univariate analyses, we observed significant associations between efflux levels and preβ-1, preβ-2,

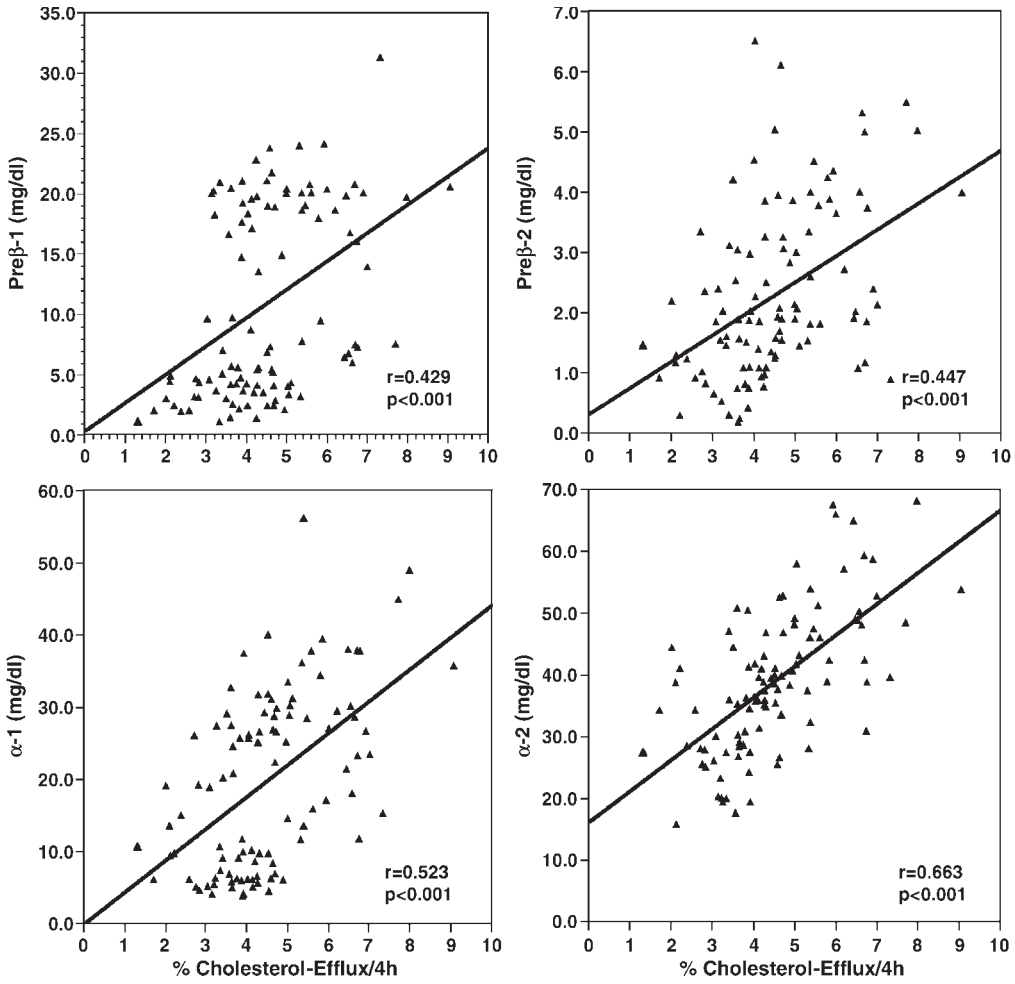

Fig. 3. Linear regression analysis of scavenger receptor class B type I (SR-BI)-mediated cholesterol efflux from Fu5AH hepatoma cells and levels of various HDL subpopulations.

α-1, α-2, preα-1, and preα-2 concentrations (**Table 3**). In multivariate analyses, the associations between SR-BI-mediated cholesterol efflux and preβ-1, α-1, α-2, and α-3 levels remained significant. There were many regression models that were equally effective in terms of the adjusted R^2 statistic. However, Mallows' C_p statistic did not obtain acceptable values until all of preβ-1, α-1, α-2, and α-3 were included in the model. When preβ-1_a and preβ-1_b were calculated separately, we found that only the smaller preβ-1_b correlated significantly with the cholesterol efflux via the SR-BI pathway (data not shown).

In a subsequent analysis, the levels of each HDL particle were plotted against ABCA1- and SR-BI-mediated cholesterol efflux and the fitness of the scatterpoints (r) was evaluated. Mean ABCA1-mediated cholesterol efflux showed a significant positive relationship with the preβ-1 level only (**Fig. 2**). In contrast, SR-BI-mediated cholesterol efflux values showed significant positive associations with four HDL subpopulations, preβ-1, preβ-2, α-1, and α-2 (**Fig. 3**). It is interesting that even though α-3 did not have statistically significant predictive capability on its own, it significantly increased the association between α-1 levels and SR-BI-mediated cholesterol efflux. α-1 and α-3 had adjusted R^2 values of 0.1840 and 0.0126, respectively, when they were in a two-predictor regression model. However, for these two HDL particles combined, the adjusted R^2 (0.4620) became higher than that for α-2 (0.4170), which had the highest single value. To determine how α-1 and α-3 together fit SR-BI-mediated efflux better than suggested by their individual contributions, we studied the three-dimensional scatterplots of α-1, α-3, and SR-BI-mediated efflux data. We rotated the chart to see the surface of scatter from different angles of the axes. This analysis indicated that including α-3 into the model made α-1 scatter tighter, improving the R^2 (**Fig. 4**).

DISCUSSION

The role of HDL in RCT has been studied extensively, but it is not completely understood. RCT involves all HDL particles, HDL-modifying factors (LCAT, cholesteryl ester transfer protein, phospholipid transfer protein, lipases, etc.), and two cell surface proteins, ABCA1, which promotes the unidirectional flux of cholesterol out of cells, and SR-BI, which enhances the bidirectional flux of FC and the unidirectional influx of CE. There are numerous reports describing how whole plasma, whole HDL fraction, or specific HDL subclasses (HDL_2, HDL_3, LpA-I, LpA-I:A-II, preβ-1, and reconstituted HDL particles with a variety of compositions) promote cholesterol efflux from cells. However, there is no information on correlations of individual HDL particles and cholesterol efflux when they are present as a mixture, as in serum.

In this study, we investigated how the entire HDL fraction promoted cholesterol efflux via the ABCA1 and SR-BI pathways and related these efflux data to the concentration of individual HDL particles, determined quantitatively by two-dimensional nondenaturing gel electrophoresis, immunoblotting, and image analysis. Our objective was to

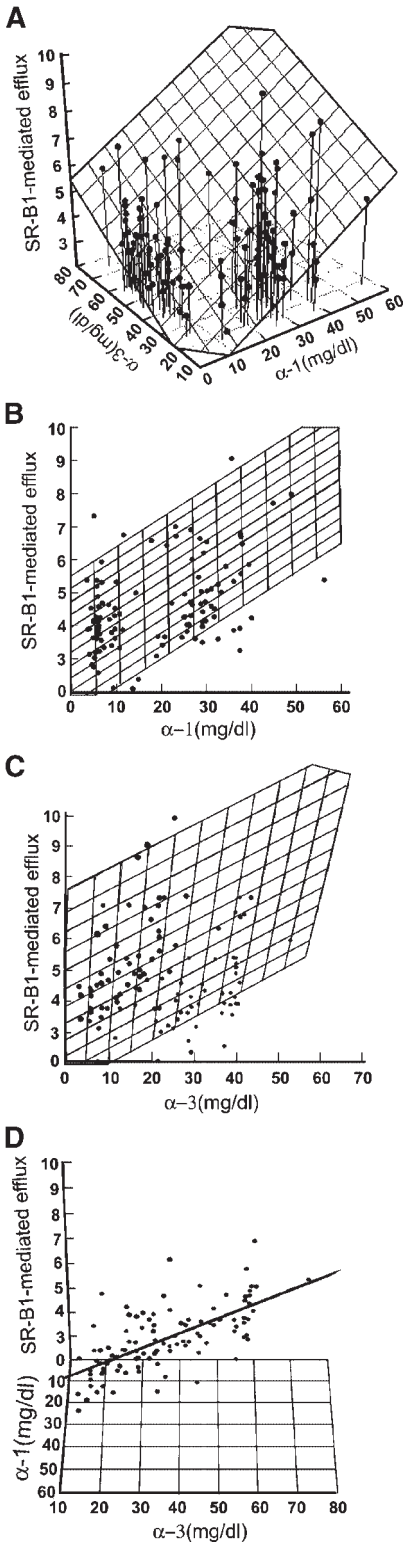

Fig. 4. Association between SR-BI-mediated cholesterol-efflux from Fu5AH hepatoma cells and levels of HDL particles (preβ-1, preβ-2, α-1, and α-2). A: Regression plane from the view of all three axes (SR-BI-mediated efflux, α-1 levels, and α-3 levels). B: Regression plane from the perspective of SR-BI-mediated efflux and α-1 levels. C: Regression plane from the perspective of SR-BI-mediated efflux and α-3 levels. D: Regression plane from the perspective of SR-BI-mediated efflux and α-1 levels when the α-3 surface is turned to horizontal. The comparison between B and D indicates that α-3 levels influence the fitness between α-1 level and SR-BI-mediated efflux.

evaluate the associations between levels of individual HDL subpopulations and ABCA1- and SR-BI-mediated cholesterol efflux.

Interestingly, HDL fractions obtained from subjects with high preβ-1 and high α-1 levels promoted the greatest ABCA1-mediated cholesterol efflux. However, the HDL fraction of subjects with low preβ-1 and high α-1 levels did not enhance cholesterol efflux via the ABCA1 pathway.

We hypothesize that factors other than HDL particle levels influence ABCA1-mediated cholesterol efflux as well. These factors may include the lipid and/or apolipoprotein composition of HDL particles, which can influence the conversion of preβ-1 to larger, more lipidated α-mobility particles. Recent data indicate that TG-rich lipoproteins inhibit ABCA1-mediated cholesterol efflux from macrophages (43). Subjects with high preβ-1 and high α-1 levels usually have lower than average total TG levels; consequently, they have lower than average TG-rich lipoprotein and HDL-TG levels. Data on ABCA1-mediated cholesterol efflux to whole serum showed the same trend: plasma samples with high preβ-1 and high α-1 levels promoted more cholesterol efflux than other samples via the ABCA1 pathway (data not shown).

In contrast to ABCA1-mediated cholesterol efflux, several individual HDL subpopulations (preβ-1, preβ-2, α-1, α-2, and α-3) were significantly associated with cholesterol efflux via the SR-BI pathway. The highest level of SR-BI-mediated cholesterol efflux was also observed in subjects with high preβ-1 and high α-1 levels. In addition to a high α-1 level, this group had the highest mean level of α-2 (33% more than the average of all subjects). Both α-1 and α-2 levels were highly correlated with SR-BI-mediated cholesterol efflux; therefore, the highest combined levels of α-1 and α-2 might be the explanation for the highest efflux level.

SR-BI has a bidirectional function by promoting both selective cholesterol uptake from HDL and cholesterol efflux to HDL. However, the method we used allowed us to determine only cholesterol efflux to HDL via the SR-BI pathway. We do not know how these data are applicable to selective cholesterol uptake from HDL. The two small, lipid-poor HDL particles (preβ-1 and α-3) probably do not contribute significant amounts of cholesterol to cells via SR-BI. We hypothesize that the two large HDL subpopulations α-1 and α-2, containing the bulk of HDL neutral lipids, are more than adequate substrate for SR-BI-mediated selective cholesterol uptake. In contrast to α-1, α-2 is an LpA-I:A-II particle, and apoA-II was reported to modulate the binding and selective lipid uptake from rHDL by SR-BI (44). This recent report indicated that A-I/A-II-rHDL had decreased binding but increased selective uptake compared with apoA-I-rHDL. However, these rHDL particles do not mirror the lipid and apolipoprotein composition of mature α-1 and α-2 particles. We believe that α-2 level is a very important factor in RCT because α-2 levels are two to six times higher than α-1 levels in normal human plasma and even higher in certain pathological conditions in which α-1 levels are negligible but α-2 levels are still close to normal (27–29).

Because the potential for selectively increasing HDL subpopulations with different drug classes is increasing, there is an urgent need for information about the role of these HDL subpopulations in modifying CHD risk. Statins and niacin primarily increase α-1 levels (28, 45). Preliminary data on apoA-I and HDL mimetics indicate that these compounds primarily increase preβ-1 levels. Our preliminary data indicate that in the Veterans Affairs HDL Intervention Trial, gemfibrozil primarily increased α-2 levels (unpublished data).

We conclude that SR-BI-mediated cholesterol efflux from hepatoma cells is significantly associated with the cholesterol-rich LpA-I α-1 and LpA-I:A-II α-2 HDL particles, whereas the ABCA1-mediated FC efflux from macrophages is significantly associated with the lipid-poor preβ-1 HDL. In this study, we measured associations between HDL particle levels and cholesterol efflux. The cause-effect relationship and the mechanism still have to be elucidated by incubating isolated HDL subpopulations with the cells.

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