Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux[1]

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Abstract Normal high density lipoprotein (N-HDL) is remodeled during acute phase (AP) reactions by the association of serum amyloid A (SAA) and the depletion of apolipoprotein (apo) A-I. To determine the impact of this remodeling on HDL function, the capacities of N-HDL and AP-HDL to associate with and promote cholesterol efflux from human monocytic THP-1 cells were compared. THP-1 cells preferentially bound AP-HDL compared with N-HDL. Examination of the AP-HDL particles bound to THP-1 cells revealed a disproportionate association of an apoSAA-enriched, apoA-I-depleted subpopulation compared with the composition of the starting material. However, N-HDL and AP-HDL promoted cholesterol efflux from THP-1 cells equally efficiently and in a dose-dependent manner. When N-HDL was experimentally remodeled with apoSAA to achieve an apoprotein composition similar to that of the preferentially bound particles, cellular cholesterol efflux was reduced by 30%. The remodelling of HDL with apoSAA during the acute phase reaction alters cholesterol efflux only when apoSAA constitutes more than 50% of the HDL protein. **—Banka, C. L., T. Yuan, M. C. de Beer, M. Kindy, L. K. Curtiss, and F. C. de Beer.** Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux. *J. Lipid Res.* 1995. **36:** 1058–1065.

Supplementary key words acute phase proteins • apolipoproteins • reverse cholesterol transport

Threats to homeostasis including injury, infection, and inflammation elicit hepatic synthesis of a variety of acute phase proteins in mammals (for review, see ref. 1). Serum amyloid A proteins (apoSAA), encoded by a multigene family (2), are major acute phase reactants in humans (3). ApoSAAs are small molecules (104 amino acids in humans) that circulate as major apolipoprotein components of high density lipoprotein (HDL) during acute phase reactions (4). No definitive function has been demonstrated for the apoSAA proteins. However, the ability of apoSAA to displace apolipoprotein (apo) A-I, the major apolipoprotein component of HDL (4), and the reduction in HDL levels during disease states in humans (4, 5), and during induced acute phase reactions in experimental animals (6, 7) has led to speculation that apoSAA may alter HDL function and metabolism. This is supported by observations that high plasma levels of apoSAA are associated with diminished activity of the plasma cholesterol esterifying enzyme, lecithin:cholesterol acyltransferase (8) and that the presence of apoSAA reduces HDL affinity for hepatocytes and enhances HDL affinity for macrophages (9). However, the impact of apoSAA on HDL-mediated cellular cholesterol efflux has not been investigated.

The removal of cholesterol from peripheral cells is an important component of reverse cholesterol transport by which excess cholesterol is directed from the periphery to the liver for catabolism (10–12). Whereas it is generally accepted that HDL is the major acceptor of cholesterol from cells, the role of the individual exchangeable apolipoproteins in this process is the subject of controversy. Particles containing apoA-I are the most efficient acceptors of cellular cholesterol; however, apoA-II and apoC also can promote cholesterol efflux (13–15). Furthermore, a dimer of an 18 amino acid peptide with no sequence homology to the apolipoproteins was reported to stimulate clearance of cholesterol from cells (16). The common feature of these diverse proteins is their amphipathic alpha helical content (16, 17). These observations have led Rothblat and coworkers (18) to propose a receptor-independent interaction between amphipathic helical motifs of apolipoproteins and specific domains of the plasma membrane as the mechanism underlying apolipoprotein-mediated cholesterol efflux. Studies by Oram and coworkers (19) sug-

Abbreviations: apoSAA, apolipoprotein serum amyloid A; apo, apolipoprotein; AP-HDL, acute phase high density lipoprotein; N-HDL, normal HDL, $N\text{-}HDL_{rf}$, refloated normal HDL; $AP\text{-}HDL_{rf}$, refloated acute phase HDL; N-HDL/AI, normal HDL remodeled with apoA-I; N-HDL/SSA, normal HDL remodeled with SAA.

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gest that the hydrolysis and mobilization of intracellular cholesteryl ester stores leading to cellular cholesterol efflux is initiated through an HDL cell surface receptor and a candidate receptor has been cloned and sequenced (20). We have recently demonstrated specific inhibition of cellular cholesterol efflux to HDL with monoclonal antibodies that bind a region containing two adjacent amphipathic helical repeats in the putative hinged domain of apoA-I (13). This suggests that specific regions of apoA-I are critical for HDL receptor binding or for HDL uptake of free cholesterol.

These observations prompted us to explore the interactions between apoSAA-containing acute-phase HDL (AP-HDL) and macrophages with emphasis on the process of cellular cholesterol efflux. Because HDL particles are dramatically remodeled by the association of apoSAA and the displacement of apoA-I during acute phase reactions, we predicted that the presence of apoSAA would inhibit cholesterol efflux to HDL. To test this hypothesis, we examined the impact of AP-HDL compared to normal HDL (N-HDL) on cell association of HDL and on the process of cholesterol efflux from human monocytic THP-1 cells.

METHODS

HDL isolation and iodination

Normal and acute phase plasma samples were drawn from the same donors before and after surgery. AP-HDL and N-HDL were prepared by sequential ultracentrifugation as previously described (4). The respective HDLs were further subfractionated into HDL_2 (d 1.063–1.13 g/ml), HDL_{3a} (d 1.130–1.155 g/ml), HDL_{3b} (d 1.155–1.18 g/ml), and HDL_{3c} (d 1.18–1.21 g/ml). Lipoproteins were iodinated (^{125}I) using a modified iodine monochloride method (4). After SDS-PAGE separation, Coomassie Blue staining, and pyridine extraction, the protein content of each apolipoprotein was calculated from standard curves of pyridine-extracted apolipoproteins (21). The specific activity of each ^{125}I-labeled apolipoprotein in N-HDL and AP-HDL was also calculated. In a typical experiment, the specific activities of apoA-I, apoA-II, and apoSAA were 411, 398, and 490 cpm/ng, respectively.

Purification of apoSAA and apoA-I

ApoSAA was purified as described (21). Briefly, AP-HDL was delipidated at −20°C with ethanol–diethyl ether 3:2 and the protein pellet was dissolved in 0.02 M Tris, 0.15 M NaCl, pH 8.4, containing 7 M urea. Dissolved apolipoproteins were applied to a column (1 × 100 cm) (Bio-Rad) of Sephacryl S-200 (Pharmacia) and eluted at room temperature in the same buffer. SDS-PAGE analysis of the fractions was performed. Pure apoSAA-containing fractions were dialyzed against 0.002 M Tris, 0.015 M NaCl, pH 8.4, lyophilized to one tenth the original volume, and stored at 4°C. ApoA-I was purified similarly except that N-HDL was used as starting material.

Preparation of apoSAA-enriched HDL

Aliquots containing 2 mg of $N\text{-}HDL_{3a}$ were incubated with 7 mg purified apoSAA (N-HDL/SAA) or apoA-I (N-HDL/AI) at 25°C for 1 h in 0.02 M Tris, 0.15 M NaCl, pH 7.4. Two-mg aliquots of $N\text{-}HDL_{3a}$ and $AP\text{-}HDL_{3a}$ were mock-incubated as controls without apolipoprotein ($N\text{-}HDL_{rf}$ and $AP\text{-}HDL_{rf}$, respectively). The density of the incubation mixtures was adjusted to 1.25 g/ml with solid potassium bromide and the HDL samples were refloated ultracentrifugally (4). Fractions containing HDL were collected and dialyzed extensively against 0.15 M NaCl, 0.002 M EDTA, pH 7.4. Protein concentrations of the particles were determined (22) and individual apolipoprotein content was established by pyridine extraction of Coomassie Blue-stained bands from SDS-PAGE as described (21).

THP-1 cell culture

THP-1 cells were obtained from the American Type Culture Collection and maintained in suspension in T-150 culture flasks (Costar, Cambridge, MA) at a cell density of 1.0–5.0 × 10^5/ml in 100 ml of RPMI-1640 containing 10% FCS (Irvine Scientific, Santa Ana, CA), 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, and 5 × 10^{-5} M β-mercaptoethanol. Cultures were maintained at 37°C in 5% CO_2. Cells were not used beyond 20 passages.

Comparative $AP\text{-}HDL_3$ and $N\text{-}HDL_3$ association with THP-1 cells

THP-1 cells (8 × 10^5) were placed in borosilicate glass tubes with 1.0 ml of serum-free RPMI-1640 culture medium containing increasing amounts of ^{125}I-labeled $N\text{-}HDL_3$ or ^{125}I-labeled $AP\text{-}HDL_3$. Triplicate samples were incubated for 1 h at 37°C, centrifuged at 4°C, and the supernatants containing unbound ^{125}I-labeled HDL were collected. Pellets were washed three times with cold PBS, 0.2% BSA at 4°C, resuspended in 1 ml of the same buffer, and transferred to clean tubes at 4°C. They were again pelleted and washed a further three times with PBS at 4°C. Cell pellets were then dissolved in 1 N NaOH for gamma counting and protein measurement (23) or in SDS-sample buffer for SDS-PAGE analysis and autoradiography. Duplicate no-cell control experiments were performed using empty tubes.

Cellular degradation of $N\text{-}HDL_3$ and $AP\text{-}HDL_3$ during the 1-h 37°C incubation was assessed in all samples as described (23). Briefly, cell-free supernatants (1 ml) were precipitated with 12% (w/v) trichloroacetic acid (TCA). The TCA-soluble material was extracted with chloroform after oxidation with H_2O_2 and counted.

Cholesterol efflux assays

Cholesterol efflux from THP-1 cells was measured by monitoring the appearance in cell culture supernatants of cholesterol synthesized from [^{14}C]acetate. This system has proven effective for quantitating both apoA-I-dependent and diffusional cholesterol efflux (13). Individual experiments were conducted under serum-free conditions by washing the cells three times and culturing them in RPMI-1640 medium in which the 10% FCS was replaced with 1% Nutridoma-HU (Boehringer Mannheim Biochemicals, Indianapolis). Cells were seeded at 1–2 × 10^5 cells in 0.5 ml/well in 24-well tissue culture dishes (Costar, Cambridge, MA). [^{14}C]acetate (New England Nuclear, Boston, MA) was added at a final concentration of 500 μM acetate with a specific activity of 5 mCi/mmol. Each 0.5 ml culture of THP-1 cells received 1.25 μCi of [^{14}C]acetate. Cholesterol acceptors (HDL or remodeled HDL) were added after a 2-day exposure of cells to [^{14}C]acetate. After a further 24-h culture period, 0.5 ml of trypsin (0.5 mg/ml trypsin, 0.2 mg/ml EDTA, Irvine Scientific, Santa Ana, CA) was added to the cultures for 5 min at 37°C to ensure dissociation of SAA-containing HDL particles from the cell surface (23). The cells and supernatants were separated by centrifugation and collected. Cells were frozen for subsequent DNA assays. Supernatants were immediately transferred to tubes containing 1.0 ml of ethanol and stored at 4°C. Supernatant lipids were subsequently extracted into hexane and separated by thin-layer chromatography on silica gel 60A plates (250 mm layer, 10 × 20 cm, Whatman, Maidstone, England) with a solvent of isopropyl ether–glacial acetic acid 96:4. ^{14}C-labeled sterol bands were identified autoradiographically, scraped, and counted in 2.0 ml of Bio Safe NA (RPI Corp., Mount Prospect, IL). The ^{14}C-labeled sterol(s) effluxed from THP-1 cells have not been analyzed by high pressure liquid chromatography and, therefore, may include synthetic precursors of cholesterol. For simplicity, the sterol(s) will be referred to as cholesterol on the basis of co-migration with a [^{3}H]cholesterol internal standard. Supernatant [^{14}C]cholesterol was corrected for recovery based on a [^{3}H]cholesterol internal standard and expressed as cpm/μg of cellular DNA.

Cellular DNA assay

All cholesterol parameters were normalized to cellular DNA content to account for variability introduced by differences in cell plating, supernatant recoveries, or extractions. Cellular DNA was measured using a colorimetric assay as described previously (24). DNA concentration was calculated from a standard curve prepared with calf thymus DNA (Sigma Chemical Co., St. Louis, MO). DNA values within each experiment varied by less than 10% suggesting that treatments with different HDL preparations for 24 h did not affect THP-1 cell division or viability.

Lipid assays

The phospholipid content of the particles was measured using a colorimetric kit (WAKO, Osaka, Japan) and calculated from a curve prepared from standards provided with the kit. The cholesterol content of the particles was measured using a fluorometric enzymatic cholesterol assay (25).

RESULTS

Effect of AP-HDL$_3$ and N-HDL$_3$ on cellular cholesterol efflux

To investigate the possible impact of apoSAA on HDL-mediated cholesterol efflux, we examined THP-1 cellular responses to N-HDL$_3$ and AP-HDL$_3$ from the same subject. The HDL$_3$ subfraction was chosen for the studies because it is the predominant apoSAA-bearing particle (4). Dividing THP-1 cells were cultured in the presence of [^{14}C]acetate for 48 h, the period required for at least one cell cycle (26), allowing for representative distribution of [^{14}C]cholesterol among the different cellular cholesterol pools. Efflux of newly synthesized [^{14}C]cholesterol was then monitored after a 24-h exposure to increasing concentrations of HDL. As illustrated in **Fig. 1,** the HDL-mediated efflux of [^{14}C]cholesterol from THP-1 cells was concentration-dependent and equivalent for N-HDL$_3$ and AP-HDL$_3$ although apoSAA accounted for 27% of the AP-HDL$_3$ protein. In both cases, 50 μg/ml of HDL protein induced a twofold increase in supernatant [^{14}C]cholesterol compared with the control. The increase in cholesterol efflux was fourfold in response to N-HDL$_3$ and AP-HDL$_3$ at 400 μg/ml. This level of efflux is comparable to that observed with total HDL (13). Previous studies with this cell system have established that appearance of [^{14}C]cholesterol in the culture supernatants reflects a loss of cholesterol mass from the cells and not just exchange of HDL cholesterol with cellular [^{14}C]cholesterol (13).

Association of AP-HDL$_3$ and N-HDL$_3$ with THP-1 cells

Figure 2 shows the association of ^{125}I-labeled N-HDL$_3$ and ^{125}I-labeled AP-HDL$_3$ with THP-1 cells. When cells were exposed to increasing concentrations of ^{125}I-labeled HDL for 1 h at 37°C, significantly more ^{125}I-labeled AP-HDL$_3$ than ^{125}I-labeled N-HDL$_3$ apolipoproteins associated with the cells at all concentrations tested. The nonspecific binding of ^{125}I-labeled N-HDL$_3$ and ^{125}I-labeled AP-HDL$_3$ did not differ significantly (data not shown).

SDS-PAGE analysis and autoradiography allowed for identification of the specific cell-associated apolipoproteins and suggested a disproportionate association of apoSAA with THP-1 cells (**Fig. 3**). Therefore, in a subsequent experiment, individual apolipoprotein bands were

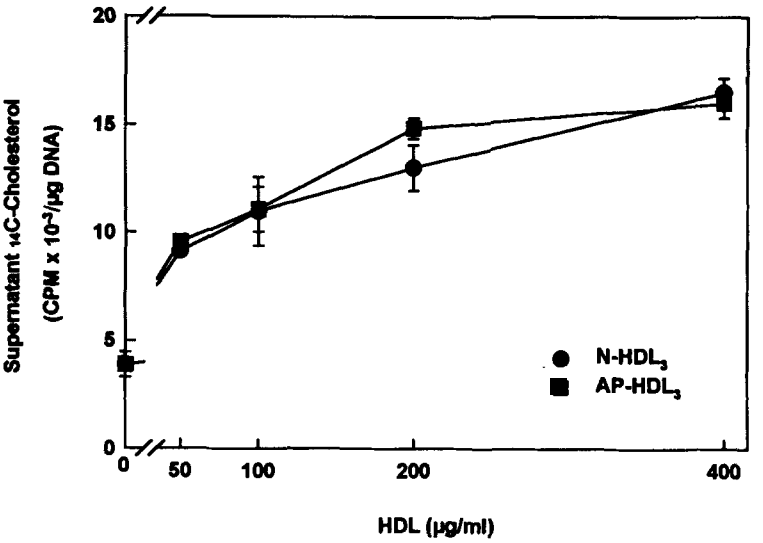

Fig. 1. Dose dependence of HDL-mediated cellular cholesterol efflux. THP-1 cells were cultured in serum-free medium at 1.5 × 10^5 cells per 0.5 ml in 24-well plates. After a 2-day exposure to [^{14}C]acetate, the cells were incubated with medium (0 mg/ml protein) or increasing concentrations of normal (N-HDL$_3$) or acute phase (AP-HDL$_3$) HDL$_3$. After 24 h, supernatant [^{14}C]cholesterol was extracted and measured as described in Methods. Values are normalized to DNA content of the cells and represent the mean ± SD of four replicate cultures.

excised and counted, and the percent contribution of each ^{125}I-labeled apolipoprotein to the total bound lipoprotein was calculated on the basis of the respective specific activities. The ratio of apoA-I to apoA-II in the cell-associated ^{125}I-labeled N-HDL$_3$ (apoA-I: 70%; apoA-II: 30%) was found to be identical to that in the starting material at all concentrations tested (**Table 1**). In contrast, the cell-associated ^{125}I-labeled AP-HDL$_3$ apolipoproteins were markedly enriched for apoSAA compared with the starting material. The apolipoprotein distribution in the ^{125}I-labeled AP-HDL$_3$ presented to the cells was apoA-I: 50%, apoA-II: 14%, and apoSAA: 36%. When 400 µg/ml ^{125}I-labeled AP-HDL$_3$ was incubated with THP-1 cells for 1 h at 37°C, the cell-associated apolipoprotein distribution was 26%, 8%, and 66%, respectively. The ratio of cell-associated AP-HDL$_3$ apolipoproteins remained the same at all concentrations tested (Table 1). Empty tubes had <1% of associated counts and after prolonged autoradiographic exposure of SDS-PAGE, the AP-HDL$_3$ material from these no-cell controls resembled the starting material, indicating that the selective apolipoprotein distribution was cell specific. After a 1-h incubation, degradation of both cell-associated N-HDL$_3$ and AP-HDL$_3$, assessed as the recovery of TCA-soluble radioactivity, was less than 2% of cell-associated ligand at all concentrations tested. At 24 h degradation increased to 4.3% for N-HDL$_3$ and 6.8% for AP-HDL$_3$, suggesting that little internalization of HDL occurred during incubations at 37°C.

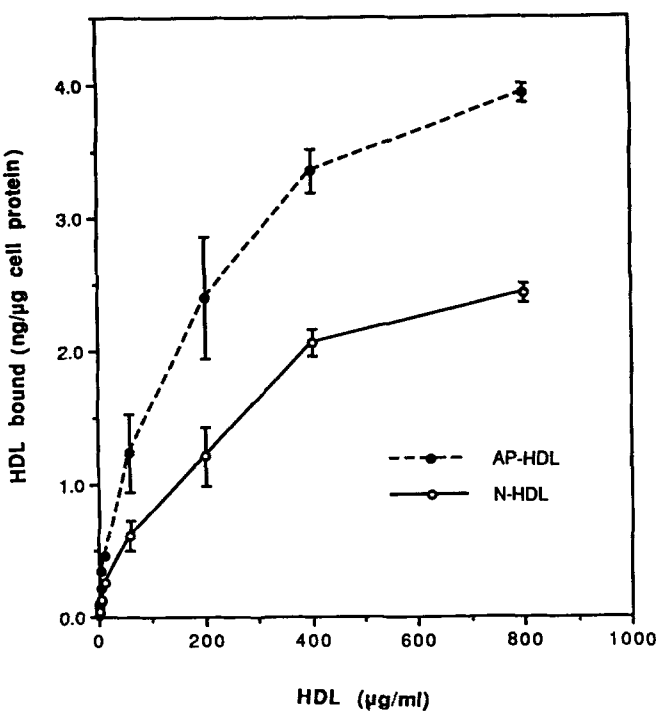

Fig. 2. THP-1 cellular association of ^{125}I-labeled N-HDL$_3$ and ^{125}I-labeled AP-HDL$_3$ apolipoprotein. THP-1 cells (8 × 10^5 tube) were exposed to increasing concentrations of ^{125}I-labeled HDL for 1 h, pelleted, washed extensively, dissolved in 1 N NaOH, and counted. Each concentration point represents mean ± SD of triplicate determinations.

Effect of SAA-containing particles on cholesterol efflux from THP-1 cells

Despite the apparent lack of inhibition of cholesterol efflux by apoSAA in initial experiments (see Fig. 1), the demonstration of selective binding of the apoSAA-rich subfraction of AP-HDL$_3$ to THP-1 cells (see Fig. 3B and Table 1) prompted us to examine the effect of HDL preparations with higher proportions of apoSAA protein. This was accomplished by remodeling N-HDL$_3$ with purified apoSAA in vitro and reisolating the particles by ultracentrifugation. ApoA-I-remodeled HDL (N-HDL/AI) and reisolated (or "refloated") N-HDL$_3$ (N-HDL$_{rf}$) and AP-HDL$_3$ (AP-HDL$_{rf}$) served as controls for the re-

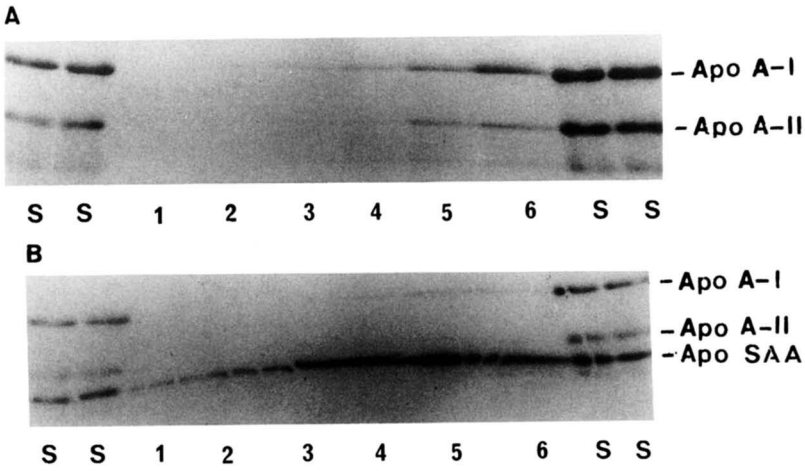

Fig. 3. Preferential cellular association of a subpopulation of apoSAA-enriched AP-HDL. Autoradiograph of THP-1 cell-associated ^{125}I-labeled HDL separated on non-reduced 4–30% gradient SDS-PAGE. Samples were from an experiment conducted as described in Fig. 2. Lanes 1–6 represent the cell-bound apolipoproteins after incubation with 2, 5, 10, 20, 60, and 200 μg of ^{125}I-labeled N-HDL$_3$ (A) or ^{125}I-labeled AP-HDL$_3$ (B) protein/ml respectively. Lanes S contain 5 μg of HDL protein from standard preparations of ^{125}I-labeled N-HDL$_3$ (A) or ^{125}I-labeled AP-HDL$_3$ (B).

modeled apoSAA particles (**Fig. 4B**). Additionally, we subfractionated the N-HDL$_3$ and AP-HDL$_3$ by gradient ultracentrifugation (4) (Fig. 4A), characterized the lipid and protein content of the particles, and tested them in the cholesterol efflux assay. Lipid and protein parameters for the preparations are summarized in **Table 2.** As previously demonstrated (4), the apoSAA-enriched particles had higher protein:cholesterol ratios than the paired non-SAA-containing controls in all cases (compare N-HDL$_{3a}$ with AP-HDL$_{3a}$, HDL/AI with HDL/SAA, etc., in Table 2). In all but one case, N-HDL$_{3c}$ versus AP-HDL$_{3c}$, in which limited material allowed only for a single assay sample, the apoSAA-containing HDLs had higher protein:phospholipid ratios than the matched N-HDL controls; however, the cholesterol:phospholipid ratios remained essentially unchanged. As expected, when these particles were added to THP-1 cells on the basis of their lipid content (60 μg/ml phospholipid), the N-HDL$_{3a}$, the "refloated" N-HDL$_{3a}$ (N-HDL$_{rf}$) and the apoA-I-remodeled particles (N-HDL/AI) induced cholesterol efflux to the same extent with a greater than sixfold increase over control (**Fig. 5**). Of the non-SAA-containing HDLs, only the N-HDL$_{3c}$ was less effective. However, in all cases the apoSAA-containing HDLs were less efficient than the matched N-HDL controls at promoting cholesterol efflux. The HDL preparation with the highest apoSAA content, N-HDL/SAA, was characterized by the greatest inhibition of cellular cholesterol efflux (30% decrease in efflux, $P < 0.001$, compared with N-HDL/AI). These results indicated that the presence of apoSAA on HDL had little impact on cellular cholesterol efflux unless apoSAA represented more than 50% of the HDL protein.

DISCUSSION

Epidemiologic data has revealed an inverse relationship between HDL levels and the risk of cardiovascular disease. This relationship applies to levels of both HDL cholesterol and apoA-I (27, 28). Any decrease in HDL or disturbance in HDL composition may compromise cholesterol homeostasis and ultimately increase the risk of atherosclerosis. The acute phase response is characterized by both a decrease in HDL (5–7) and a displacement of

TABLE 1. Apolipoprotein composition of cell-associated normal and acute phase HDL

		Cell-Associated N-HDL$_3$[a] Concentration Added				Cell-Associated AP-HDL$_3$[b] Concentration Added		
	N-HDL$_3$[b]	5 μg/ml	60 μg/ml	400 μg/ml	AP-HDL$_3$[a]	5 μg/ml	60 μg/ml	400 μg/ml
	%		%		%		%	
ApoA-I	70	70	74	73	50	27	32	26
ApoA-II	30	30	26	27	14	12	10	8
ApoSAA					36	61	58	66

[a]Apolipoprotein composition of the HDL$_3$ starting material presented to the THP-1 cells.
[b]Apolipoprotein composition of the HDL$_3$ associated with THP-1 cells after a 1-h incubation at 37°C at the concentration indicated.

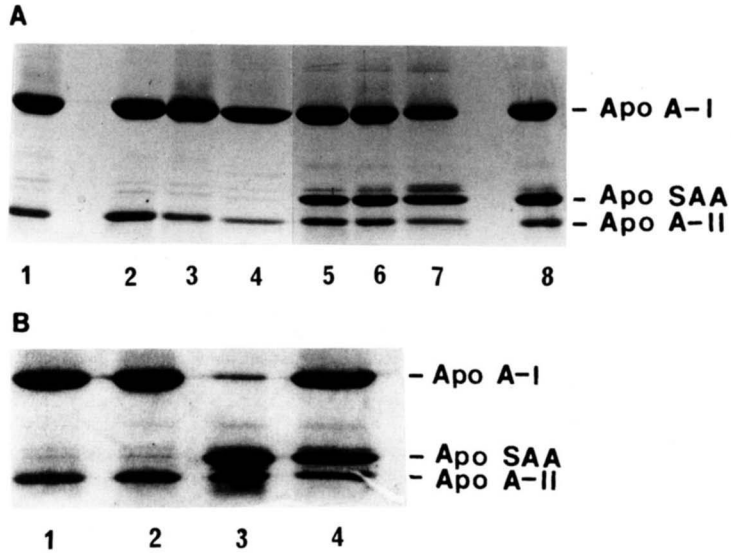

Fig. 4. Electrophoretic characterization of HDL particles used in cellular cholesterol efflux assays. Photographs of Coomassie Blue-stained reduced 4–30% gradient SDS-PAGE. Panel A: HDL_3 ultracentrifugally subfractionated. Lane 1, N-HDL standard; lane 2, N-HDL_{3a}; lane 3, N-HDL_{3b}; lane 4, N-HDL_{3c}; lane 5, AP-HDL_{3a}; lane 6, AP-HDL_{3b}; lane 7, AP-HDL_{3c}; and lane 8, AP-HDL_3 standard. Panel B: remodeled apoSAA-enriched and control particles. Lane 1, N-HDL_{3a} refloated (rf); lane 2, apoA-I-enriched N-HDL_{3a} (N-HDL/AI); lane 3, apoSAA-enriched N-HDL_{3a} (N-HDL/SAA); and lane 4, AP-HDL_3 refloated (rf).

apoA-I by apoSAA on HDL (4). The importance of these changes in HDL may be reflected in the clinical observation that patients who experience these acute phase changes on an ongoing basis, such as those with rheumatoid arthritis, have a high incidence of cardiovascular disease (29).

We have recently demonstrated that HDL-mediated cholesterol efflux from peripheral cells can be inhibited in part by antibodies specific to apoA-I (13). Therefore, we predicted that displacement of apoA-I by apoSAA on HDL from acute phase patients could decrease the effectiveness of the HDL in promoting cellular cholesterol efflux. The data presented here indicate that HDL efficiency in promoting cholesterol efflux was compromised only when the apoSAA content of the particles exceeded 50% of the total HDL protein.

The finding that THP-1 cells selectively bound an apoSAA-enriched, apoA-I-depleted subpopulation of particles from AP-HDL_3 confirmed similar findings of selective AP-HDL_3 binding to neutrophils (23) and macrophages (9). Because the ratio of AP-HDL_3 apolipoproteins bound to cells remained the same at all concentrations tested, this selective binding is thought to reflect the preferential binding of a particular apoSAA-enriched subset of HDL particles rather than surface remodeling of the cell-associated AP-HDL_3 or transfer of apoSAA to the cell surface. Although significantly more AP-HDL_3 apolipoprotein was found associated with the cells at each concentration, the apoSAA-enriched particles were substantially more dense than the mean HDL (Table 2). Thus the number of particles bound may have been very similar despite the increase in protein bound. The presence of apoSAA appears to negatively influence the cell association of apoA-I (Fig. 3) suggesting that apoSAA may bind the same cell surface site as apoA-I (and apoA-II). However, neither the selective association of the apoSAA-

TABLE 2. Apolipoprotein and lipid distribution among HDL particles

Particles	ApoA-I	ApoA-II	SAA	Phospholipid	Cholesterol
	% of total protein			*μg total cholesterol/mg protein*[a]	
N-HDL_{3a}	71	29		608 ± 14	125 ± 11
AP-HDL_{3a}	56	17	27	570 ± 13	102 ± 2
N-HDL_{3c}	82	18		331[b]	81 ± 1
AP-HDL_{3c}	39	6	55	411[b]	56 ± 3
N-HDL_{rf}	69	31		636 ± 1	151 ± 35
AP-HDL_{rf}	58	17	25	589 ± 39	131 ± 16
N-HDL/AI	73	27		608 ± 14	149 ± 11
N-HDL/SAA	4	10	86	383 ± 39	70 ± 8

Abbreviations: N-HDL_{3a}, normal HDL_{3a}; AP-HDL_{3a}, acute phase HDL_{3a}; N-HDL_{3c}, normal HDL_{3c}; AP-HDL_{3c}, acute phase HDL_{3c}; N-HDL_{rf}, "refloated" normal HDL_3; AP-HDL_{rf}, "refloated" acute phase HDL_3; N-HDL/AI, apoA-I remodeled normal HDL_3; N-HDL/SAA, apoSAA remodeled normal HDL_3.

[a]Values given as means ± SD.

[b]Sample limitation resulted in single determinations.

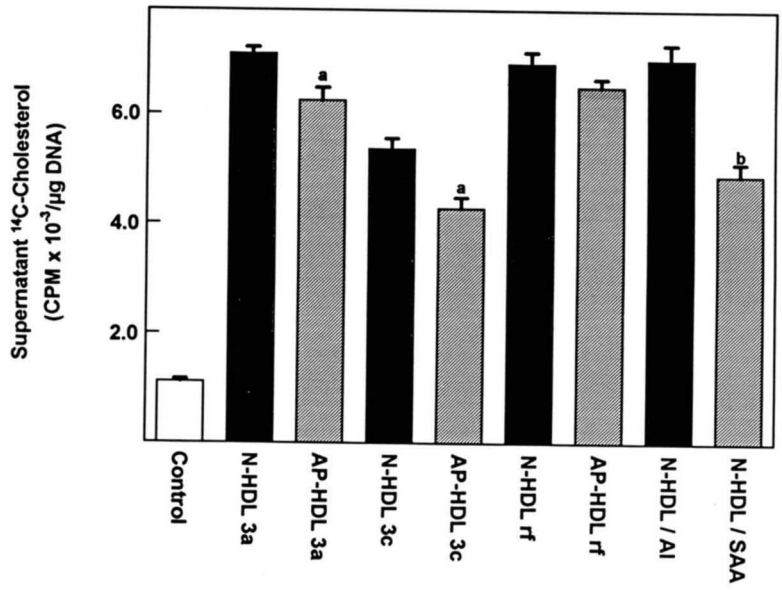

Fig. 5. Effect of apoSAA-poor and apoSAA-enriched particles on cholesterol efflux from THP-1 cells. THP-1 cells were cultured, exposed to HDL particles (60 µg/ml phospholipid), and supernatant [^{14}C]cholesterol was quantitated as described for Fig. 1. Values are normalized to DNA content of the cells and represent the mean ± SD of four replicates. Cells were exposed for 24 h to medium alone (open bar), normal and acute phase HDL subpopulations 3a and 3c, reisolated normal and acute phase HDL_{3a} (rf) or normal HDL_{3a} remodeled with apoA-I or apoSAA. a) $P < 0.01$ compared with matched control; b) $P < 0.001$ compared with matched control.

enriched particles nor the concomitant decrease in apoA-I binding resulted in dramatic reduction of cellular cholesterol efflux.

Several interpretations of the cholesterol efflux data are relevant, the most obvious being that HDL-mediated cellular cholesterol efflux is dependent upon the presence of apoA-I and, therefore, will occur less efficiently when the number of apoA-I molecules per particle drops below a certain threshold. However, particles that contained as little as 4% apoA-I promoted cholesterol efflux only 30% less efficiently than $N\text{-}HDL_3$ (see N-HDL/SAA, Table 2 and Fig. 5). Even within a narrow density range, HDL particles are polydisperse (4) making it impossible to estimate accurately the number of molecules of any individual apolipoprotein per HDL particle. However, the molecular mass of apoA-I is 2.3 times that of apoSAA, and the molar ratio of apoSAA to apoA-I in the N-HDL/SAA preparation was 50:1. Therefore, few of the particles in this population contain apoA-I. Our data suggest that, like apoA-II and apoC-III, apoSAA can promote cholesterol efflux although less efficiently than apoA-I.

The impact that these findings may have on the development of atherosclerosis during the chronic acute phase situation remains to be established. Although apoSAA can constitute up to 80% of HDL apolipoproteins in extreme circumstances (30), it rarely exceeds 50%, the concentration at which cellular cholesterol efflux can be impaired.

Our data support the contention that a number of HDL apolipoproteins can share the capacity to associate with cells and to mediate cholesterol efflux. This sharing of function is most likely based on similarities in the tertiary structure of the proteins and reflects the common characteristic of alpha amphipathic helical domains.

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