Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice

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Abstract Serum amyloid A (SAA) is an acute phase reactant that can become the predominant apolipoprotein of high density lipoprotein (HDL) during severe inflammatory states. However, the function of SAA is unknown. To study the ability of SAA to form HDL in the absence of apolipoprotein A-I, we expressed the mouse SAA pI 6.15 (CE/J) isoform in apolipoprotein A-I knock-out (apoA-I (−/−)) mice using a recombinant adenovirus. As a control, apoA-I (−/−) mice were injected with an adenovirus expressing human apoA-I. High level expression of plasma SAA was obtained in the absence of any endogenous acute phase SAA production. SAA expression increased plasma HDL cholesterol levels about 2-fold, but to a lesser extent than the expression of apoA-I (about 10-fold). The HDL particles isolated by density ultracentrifugation from SAA-expressing mice were heterogeneous in size and composition and rich in free cholesterol as well as apoE and apoA-IV. Of the SAA expressed in the plasma, only a small fraction (4%) was associated with HDL particles in contrast to expressed apoA-I, of which 62% was associated with HDL. We conclude that SAA is unable to substitute for apoA-I in HDL particle formation.—**Webb, N. R., M. C. de Beer, D. R. van der Westhuyzen, M. S. Kindy, C. L. Banka, K. Tsukamoto, D. J. Rader, and F. C. de Beer.** Adenoviral vector-mediated overexpression of serum amyloid A in apoA-I-deficient mice. *J. Lipid Res.* 1997. **38:** 1583–1590.

Supplementary key words apolipoprotein A-I • high density lipoprotein • acute phase

The acute phase response is characterized by a number of systemic metabolic changes that rapidly occur after tissue injury or inflammation (1). Marked changes in plasma lipids and lipoprotein metabolism are well-characterized components of this response (2). High density lipoprotein (HDL) in particular is altered during the acute phase when cytokines greatly stimulate production of the acute phase members of the serum amyloid A protein (SAA) family (3). Acute phase SAA associates mainly with HDL_3 particles and increases the particle radius (3). This is likely the result of an increased shell width due to the manner in which SAA (as opposed to apoA-I) associates with the particle (3). Analysis of such particles, isolated by ultracentrifugation, revealed polydispersity with respect to apolipoproteins such that denser particles contained the highest content of SAA. In extreme acute phase cases high density HDL particles virtually devoid of apoA-I can be found (4). This situation can be mimicked in vitro by the interaction of pure SAA with normal HDL_3, resulting in particles with up to 80% of the apolipoprotein being SAA (3). It has been established that SAA-bearing acute phase HDL binds more avidly to neutrophils (5) and macrophages (6, 7) than normal HDL. Analysis of cell-associated apolipoproteins after acute phase HDL binding indicated a disproportionately high concentration of SAA, raising the question whether a certain subset of SAA-enriched particles preferentially bind to cells (5).

Over the last years, immunological techniques have uncovered a polydispersity of particles in the HDL density range that differ in apolipoprotein composition (8). These apolipoproteins share an amphipathic alpha helix that comprises a multi-functional structural motif (9). The question arises as to whether these apolipoproteins are capable of forming HDL particles per se or whether their association with HDL is the end result of metabolic activities. In this paper we studied whether SAA, the only apolipoprotein apart from apoA-I that can comprise the major apolipoprotein constituent of

Abbreviations: SAA, serum amyloid A; apoA-I, apolipoprotein A-I; HDL, high density lipoprotein; Ad-SAA, recombinant adenovirus containing mouse serum amyloid A (CE/J isoform) cDNA; Ad-apoAI, recombinant adenovirus containing human apolipoprotein A-I cDNA; LPS, lipopolysaccharide; LCAT, lecithin:cholesterol acyltransferase.

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HDL, can substitute for apoA-I in apoA-I-deficient mice (apoA-I (−/−)) (10). Conventional inflammatory induction of SAA through cytokines will not only induce endogenous SAA production, but also a variety of metabolic effects. To study the effect of SAA alone on lipoproteins and lipid profiles, we developed an adenoviral vector capable of overexpressing an acute phase SAA isotype in vivo. This vector produced high levels of SAA comparable to levels generated in a LPS-induced acute phase response. This approach allowed for the study of the influence of SAA on lipoprotein profiles in the apoA-I (−/−) mouse in the absence of other acute phase responses.

EXPERIMENTAL PROCEDURES

Animals

Homozygous apoA-I-deficient mice (apoA-I (−/−)) (C57BL/6J-apoa1tm1Unc) and C57BL/6 mice were obtained from Jackson Laboratories, Bar Harbor, ME. An acute phase response was elicited by intraperitoneal injection of 10 μg lipopolysaccharide (LPS) *Escherichia coli 0111:B4*) (Difco Laboratories, Detroit, MI). Expression of human apoA-I or mouse SAA (pI 6.15) was achieved by tail-vein injection of recombinant virus. EDTA anti-coagulated blood was collected by cardiac puncture from metofane-anesthetized animals.

Adenoviral vectors expressing CE/J SAA and human apoA-I

CE/J SAA coding sequences were amplified from a cDNA clone (11) using the oligonucleotide primers 5′-GGAAGCTTGGATGAAGCTACTCACCAGCCTG (forward primer) and 5′-GGGGTACCAACACAGCCTTCT GAACT (reverse primer). The amplified fragment was digested with *Kpn* I and *Hind* III and inserted into an adenovirus expression vector pAdCMVlacZ (12) containing the cytomegalovirus (CMV) immediate early enhancer-promoter element to yield pAd-SAA. The pAd-SAA plasmid was co-transfected (using calcium phosphate) into 293 cells with AdCMVlacZ viral DNA. For the construction of Ad-apoAI, an *Acc* I, *Hind* III fragment from the apoA-I cDNA (kindly provided by J. Breslow, Rockefeller University) was inserted into the shuttle vector and similarly co-transfected. Twenty four hours after transfection the cells were overlaid with 0.8% noble agar in Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 2% fetal bovine serum and 12.5 mM $MgCl_2$. Approximately 10 days after transfection, agar plugs containing individual viral plaques were picked and virus eluted from the plugs was used as inoculum to infect 293 cells. The presence of the CE/J or apoA-I insert fragment in candidate viral isolates was verified by the polymerase chain reaction. Recombinant adenoviruses were subjected to another round of plaque purification prior to expansion in 293 cells and were purified by density gradient CsCl ultracentrifugation followed by chromatography on a 10 ml Econo-Pac® 10 DG column (Bio-Rad, Hercules, CA). The infectious titer of viral stocks, determined by plaque assay on 293 cells, ranged from 5×10^{10} to 5×10^{11} plaque forming units/ml.

Preparation of HDL

HDL was isolated from plasma essentially as described (13). Briefly, plasma density was adjusted to 1.09 g/ml with solid KBr and then centrifuged for 5.3 h at 242,000 *g* in a VTi90 rotor (Beckman Instruments, Palo Alto, CA) at 10°C. The infranatants containing HDL were collected, the density was readjusted to 1.21 g/ml, and the mixture was recentrifuged for 9.4 h at 242,000 *g* in the same rotor. HDL was collected from the top of the tube and extensively dialyzed against 150 mM NaCl, 0.1% EDTA, pH 7.4. Alternatively, isolation of HDL from FPLC fractions was achieved by pooling three fractions, adjusting the density to 1.21 g/ml, and centrifuging as above. The HDL collected was dialyzed against 15 mM NaCl, 0.01% EDTA, pH 7.4. Samples were freeze-dried and resuspended in SDS sample buffer.

Electrofocusing

Plasma samples (7 μl) were delipidated with 0.5 ml chloroform–methanol 2:1 (v/v) (14) and the delipidated apolipoproteins were suspended in 1% (w/v) sodium decyl sulfate (Eastman Kodak Co., Rochester, NY), 7 M urea, 5% (v/v) 2-mercaptoethanol. Samples were electrofocused on ultrathin acrylamide gels containing 20% (v/v) ampholines, pH 3–10, 40% (v/v) ampholines, pH 4–6.5, and 40% (v/v) ampholines, pH 7–9 (15).

Immunochemical analysis

Samples were separated on reducing SDS-PAGE (5–20% acrylamide) and electroblotted onto 0.2 μM pore-size nitrocellulose membranes (Schleicher and Schuell, Keene, NH) whereas samples on electrofocused gels were pressure-blotted overnight at room temperature (13).

Membranes were soaked in 25 mM Tris/HCl, pH 8.3, 192 mM glycine, 15% (v/v) methanol. After pressure-blotting the membrane was blocked for 16 h at 4°C in 5% (w/v) non-fat dry milk in phosphate-buffered saline (PBS) containing 2% BSA. Apolipoproteins were identified with one of the following antibodies: rabbit anti-mouse SAA, rabbit anti-human apoA-I (gift from A. J.

Lusis, UCLA), a cross-reacting goat anti-rat apoE (Atlantic Antibodies, Stillwater, MN), and rabbit anti-rat apoA-IV (gift from Dr. K. H. Weisgraber, Gladstone Institute, San Francisco).

Gel filtration chromatography of plasma lipoproteins

Plasma samples (370 μl) were chromatographed by FPLC on two Superose 6 columns (Pharmacia LKB Biotechnology Inc.) linked in series (16, 17). Plasma from three mice were pooled, centrifuged twice for 10 min at 10,000 rpm, first at room temperature and then at 4°C before being applied to the column at a flow rate of 0.5 ml/min. The elution buffer was 150 mM NaCl, 10 mM Tris/HCl, pH 7.4, and 0.5-ml fractions were collected.

Lipid analysis

Lipid analyses were performed on FPLC fractions or plasma samples from animals that had been fasted for approximately 7 h. Total and free cholesterol as well as triglycerides were determined enzymatically (WAKO Chemicals, Richmond, VA and Sigma Chemical Company, St. Louis, MO). HDL cholesterol was measured enzymatically after precipitation of LDL and VLDL by heparin and manganese (WAKO Chemicals, Richmond, VA).

Apolipoprotein quantitation

Apolipoproteins were quantified by electrophoresing samples (plasma or HDL) in a reducing 5–20% acrylamide SDS gel and electroblotting the samples onto nitrocellulose. Proteins of interest were identified with appropriate antibodies and visualized through chemiluminescence (Amersham). Films were analyzed by densitometric scanning (Molecular Dynamics, Sunnyvale, CA). A standard curve was obtained by electrophoresing purified apolipoproteins simultaneously. SAA levels in FPLC fractions were measured using an ELISA kit according to the manufacturer's instructions (Biosource International, Inc., Camarillo, CA).

Lecithin:cholesterol acyltransferase assays

The lecithin:cholesterol acyltransferase (LCAT) assay was based on a modification of the technique of Wallentin and Virkrot (18). The assay depends solely on endogenous plasma LCAT enzyme and substrate. [^{14}C]cholesterol was stabilized in phosphate buffer (0.2 M, pH 7.4) containing 5% heat-inactivated, fatty acid-free human serum albumin. Albumin containing 0.17 μCi [^{14}C]cholesterol was added to 100 μl of mouse plasma in the presence of 2 mM 5,5 dithiobis-2-nitrobenzoic acid (DTNB, an LCAT inhibitor). After a 4-h incubation at 37°C, mercaptoethanol (10 mM) was added to reverse the DTNB inhibition and the reaction mix was incubated at 37°C. The LCAT reaction was stopped by the addition of 1 ml of ethanol. Cholesterol and cholesteryl ester were extracted and separated by thin-layer chromatography (TLC) as described previously (19). Agarose gel analysis of the plasma samples after the 4-h incubation with albumin stabilized [^{14}C]cholesterol indicated that in all samples approximately 60% of labeled cholesterol remained associated with albumin and the remainder associated with lipoproteins (data not shown).

RESULTS

High level expression of SAA and apoA-I in apoA-I (−/−) mice was achieved by adenovirus-mediated gene targeting. **Figure 1** shows the expression of the mouse SAA in the plasma of apoA-I (−/−) mice 3 days after injection of 1×10^{11} particles Ad-SAA (lanes 1–3). This time (3 days) was chosen based on time course experiments using 1×10^{11} Ad-SAA particles. SAA plasma levels peaked at 24 h after infection, were unchanged at 3 days, but decreased approximately 50% at 5 days (data not shown). HDL cholesterol levels peaked at 3 days and decreased approximately 40% at 5 days post-injection. At this dose of Ad-SAA, the level of plasma SAA (approximately 760 μg/ml) was comparable to the level observed in mice in which an acute phase was induced by LPS (lanes 4–6). An equivalent dose of Ad-apoAI produced high levels (approximately 2.8 mg/ml) of apo-AI in the apoA-I (−/−) mice (lanes 7–9).

Immunoblot analysis of plasma SAA after isoelectric focusing demonstrated that the only SAA expressed in the Ad-SAA-treated animals was the pI 6.15 (CE/J) isoform (**Fig. 2,** lanes 13–17). In contrast, the SAA expressed in the LPS-treated animals corresponded predominantly to the endogenous inducible mouse SAA_1 and SAA_2 acute phase isoforms (lanes 7–11). The minor band at pI 6.15 in lanes 7–11 is an isolectric focusing artefact. Thus, the SAA produced in the Ad-SAA-treated mice was not the result of a general acute phase response.

The effects of SAA and apoA-I expression on plasma lipids in the apoA-I (−/−) mice are shown in **Table 1.** The low HDL cholesterol level in apoA-I (−/−) mice (8.8 ± 2 mg/dl) was increased about 2-fold in animals treated with 1×10^{11} particles Ad-SAA (21 ± 7 mg/dl). A similar increase in HDL cholesterol was observed in LPS-treated mice. Plasma total cholesterol levels were raised to a similar extent by these two treatments. Plasma triglycerides were not significantly altered by treatment with either Ad-SAA or LPS. Treatment with

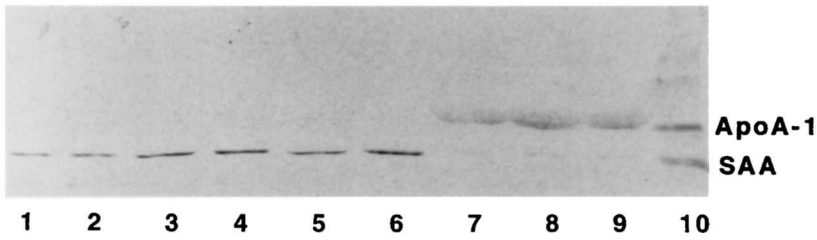

Fig. 1. Adenovirus-mediated expression of SAA and apoA-I in apoA-I (−/−) mice. Three apoA-I (−/−) mice were injected in the tail vein with 1×10^{11} particles of Ad-apoAI (lanes 7–9) or Ad-SAA (CE/J isoform) (lanes 1–3). Plasma was collected after 3 days. For comparison, apoA-I (−/−) mice were injected with 10 μg LPS (lanes 4–6) and plasma was collected 24 h post-injection. Plasma proteins from individual mice were separated by SDS-PAGE (5–20% acrylamide gradient) and subjected to immunoblotting using a mixture of rabbit anti-mouse SAA and rabbit anti-human apoA-I antibodies. Lane 10 contains plasma from LPS-treated wild-type mice.

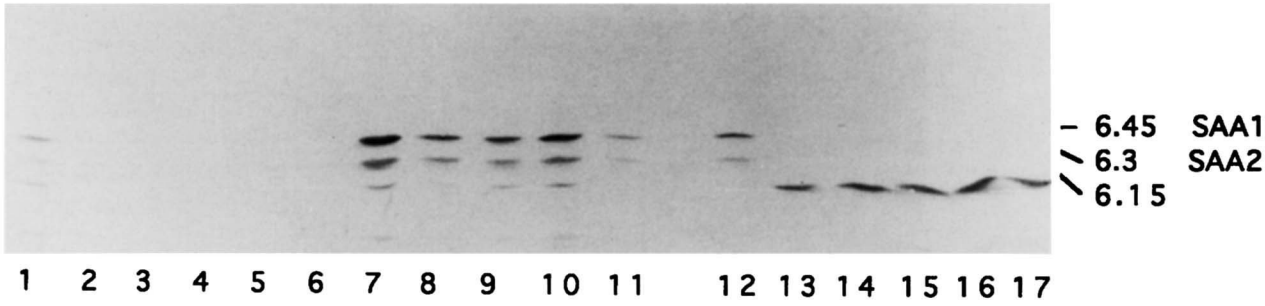

Fig. 2. Isoform-specific expression of SAA produced by Ad-SAA treatment. Plasma was obtained from apoA-I (−/−) mice 3 days after injection with 1×10^{11} particles Ad-SAA (lanes 13–17) or 24 h after infection with 10 μg LPS (lanes 7–11). Control animals (lanes 2–6) received no injection. Plasma was subjected to isoelectric focusing and immunoblotting with anti-mouse SAA. Lanes 1 and 12 contained plasma from LPS-treated wild-type mice. The minor band at pI 6.15 in lanes 7–11 is an isoelectric focusing artefact.

TABLE 1. Plasma lipids in apoA-I(−/−) mice treated with Ad-SAA, Ad-apoAI, or LPS

Treatment	n	Total Cholesterol	HDL Cholesterol	TG
		mg/dl		
No treatment	6	21 ± 2[a]	8.8 ± 2[a]	31 ± 6
Ad-SAA (1×10^{11})	6	36 ± 4[b]	21 ± 7[b]	54 ± 20
LPS	5	44 ± 4[b]	25 ± 7[b]	31 ± 4
Ad-apoAI (1×10^{11})	3	155 ± 18[c]	99 ± 11[c]	ND
Ad-apoAI (0.5×10^{11})	3	78.4 ± 2[d]	58.7 ± 2[d]	55.0 ± 4

All values are expressed in mg/dl (mean ± SE). Values marked with different letters are significantly different by analysis of variance ($P < 0.002$). The dose of virus (in particles) and the number of mice for each treatment are indicated. LPS, lipopolysaccharide; ND, not determined.

an equivalent dose of Ad-apoAI increased total cholesterol and HDL cholesterol levels about 7- and 11-fold, respectively. The HDL cholesterol level achieved by Ad-apoAI treatment (99 ± 11 mg/dl) exceeds that of normal C57BL/6 mice (16). At this dose, the level of plasma apoA-I (2.8 mg/ml) was approximately 3-fold higher than the level of SAA (0.76 mg/ml) in the Ad-SAA-treated mice. To determine whether the difference in HDL cholesterol levels could be attributed to the difference in the apolipoprotein concentration produced by Ad-SAA and Ad-apoAI, we treated apoA-I (−/−) mice with a lower dose (0.5×10^{11} particles) of Ad-apoAI. At this dose of Ad-apoAI, apoA-I plasma levels were similar to SAA levels produced in Ad-SAA-treated mice (0.73 mg/ml). However, unlike SAA, this level of expression of apoA-I in the apoA-I (−/−) mice restored HDL cholesterol to approximately normal levels (16).

HDL was isolated by sequential density flotation (d 1.09–1.21 g/ml) from untreated apoA-I (−/−) mice and mice treated with Ad-SAA, Ad-apoAI, or LPS. Plasma and HDL apolipoprotein levels were measured by quantitative immunoblotting to determine the yield of SAA and apoA-I on HDL (not shown). Whereas a large portion (62%) of plasma apoA-I was found to be associated with the isolated HDL, only a small fraction (4%) of SAA was recovered with the HDL fraction. The bulk of SAA in the plasma is therefore not associated

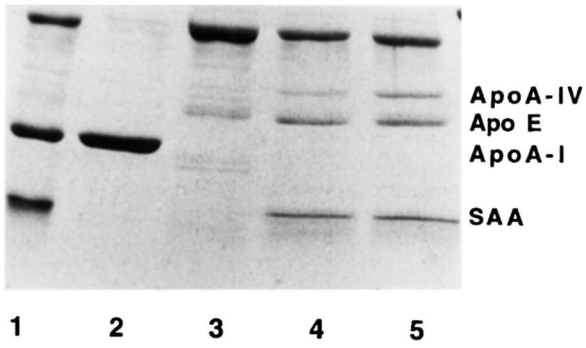

Fig. 3. Apolipoprotein composition of HDL. HDL (d 1.09–1.21 g/ml) was isolated by sequential ultracentrifugation from plasma of untreated apoA-I (−/−) mice (lane 3), or apoA-I (−/−) mice treated with Ad-apoAI (lane 2), LPS (lane 4), or Ad-SAA (lane 5). Apolipoproteins (5 μg) were separated by reducing SDS-PAGE and visualized by Coomassie blue. Lane 1 contained HDL from LPS-treated wild-type mice. The positions of apoA-IV (46 kD), apoE (34 kD), apoA-I (28 kD) and SAA (12 kD) are shown.

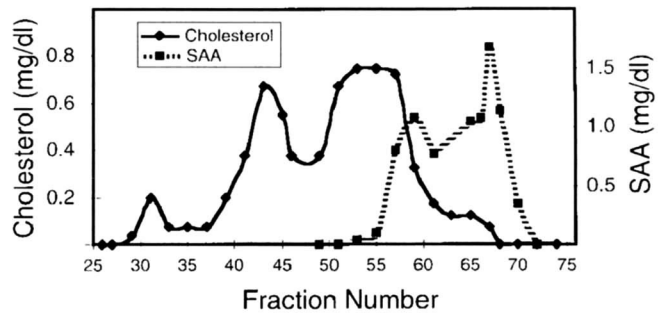

Fig. 5. FPLC fractionation of plasma from Ad-SAA-treated mice. Plasma from Ad-SAA-infected apoA-I (−/−) mice was fractionated on two Superose 6B columns in series, and 0.5-ml fractions were collected. Cholesterol and SAA determinations of individual fractions were carried out as described in Experimental Procedures.

with HDL or is associated very loosely and dissociated during ultracentrifugation in high salt.

The HDL apolipoprotein composition was analyzed by SDS-PAGE (**Fig. 3**). No apoA-I was observed in the HDL fraction isolated from apoA-I (−/−) mice (lane 3), whereas the predominant band in the Ad-apoAI-treated animals (lane 2) was, as expected, apoA-I. In the mice treated with Ad-SAA (lane 5) the predominant apolipoproteins were SAA, apoE, and apoA-IV. ApoA-II was a very minor component (<5%) of HDL isolated from Ad-SAA-treated mice. A similar apolipoprotein composition was seen for LPS-injected animals (lane 4). The upper band in the HDL samples corresponds to albumin that was not fully removed during the ultracentrifugation steps.

In view of the prominence of apoE and apoA-IV in floated HDL, plasma levels of these apolipoproteins in normal C57BL/6J, apoA-I (−/−), and Ad-SAA-treated apoA-I (−/−) mice were compared by immunoblot analysis (**Fig. 4**). Densitometric scanning of the immunoblot showed that the plasma apoE level in the apoA-I (−/−) mice was 5-fold higher compared to C57BL/6J mice, and this level was increased a further 2-fold as a result of Ad-SAA treatment. ApoA-IV plasma levels were similar in all of the mice (Fig. 4).

To further analyze the lipoproteins in apoA-I (−/−) mice treated with Ad-SAA, plasma was collected 72 h post-injection and separated by gel filtration on Superose 6B. As shown in **Fig. 5,** cholesterol eluted as a broad peak in the HDL region (fractions 50–62). SAA eluted as two peaks, with the first SAA peak partly overlapping the HDL cholesterol peak. The second major SAA peak chromatographed later than the HDL peak.

The HDL apolipoprotein distribution was studied by making four pools across the HDL cholesterol peak. HDL was floated from these pools and analyzed by SDS-PAGE (**Fig. 6**). Fractions were shown to be heterogeneous, as evidenced by apoE predominantly eluting in the early fractions, and SAA and apoA-IV in the late fractions. Again, only a small portion of the SAA present in the HDL fractions was recoverable by flotation.

Lipid analysis of the HDL fractions showed that treatment with Ad-SAA (and also LPS) increased the HDL cholesterol level in plasma, although to a markedly lesser degree than Ad-apoAI treatment (**Table 2**). A fea-

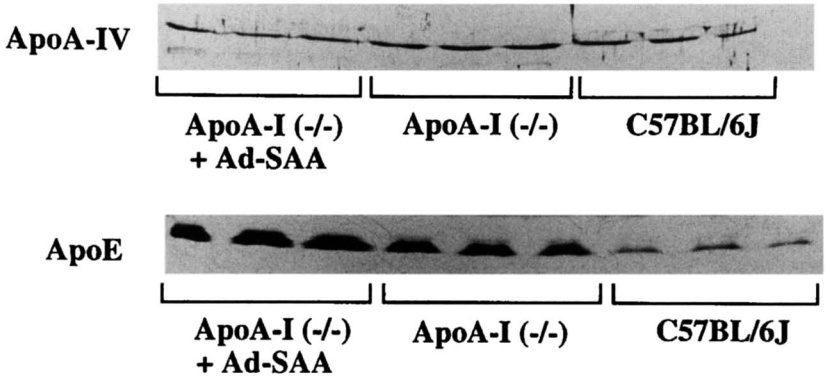

Fig. 4. ApoA-IV and apoE analyses. Plasma was collected from each of three C57BL/6J, apoA-I (−/−), and Ad-SAA-injected apoA-I (−/−) mice. A 4-μl aliquot of the pooled samples was run in triplicate on reducing SDS-PAGE, transferred to nitrocellulose, and immunoblotted with anti-apoA-IV or anti-apoE, as indicated.

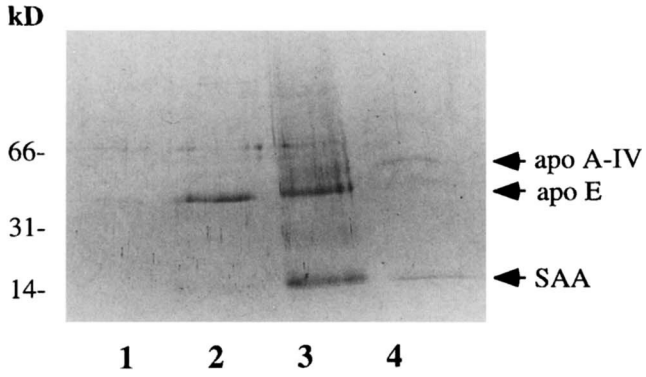

Fig. 6. Apolipoprotein composition of FPLC fractions. FPLC fractions (Fig. 5) were pooled and refloated as described in Experimental Procedures. All of the protein in each of the samples was separated by SDS-PAGE and visualized by Coomassie staining. Lane 1, fractions 49–51; lane 2, fractions 52–54; lane 3, fractions 55–57; lane 4, fractions 58–60.

TABLE 2. Lipid composition of HDL isolated from plasma pooled from three apoA-I(−/−) mice injected with 1×10^{11} particles Ad-SAA, Ad-apoAI, or LPS

Treatment	Total Cholesterol	FC	CE	TG
		mg/dl		
No treatment	8.0	3.5	ND	ND
Ad-SAA	20	14	6.0	8.9
LPS	22	10	12	7.3
Ad-apoAI	129	44	85	16

ture of the HDL isolated from Ad-SAA-treated mice was a high fraction of unesterified cholesterol (70%) compared to HDL from Ad-apoAI-treated animals (34%). To determine the possible effect of SAA expression on LCAT activity in the apoA-I (−/−) mice, endogenous LCAT activity in the plasma of untreated and Ad-SAA-treated mice was measured. Ad-SAA treatment resulted in an approximately 2.5-fold reduction in LCAT activity in the apoA-I (−/−) mice (data not shown).

DISCUSSION

The mouse acute phase SAAs (SAA_1 and SAA_2) are the products of two genes that resulted from gene reduplication and differ from each other at 9 of 103 amino acids (20, 21). The SAA expressed in the CE/J mouse strain is an exception in that gene reduplication did not occur, and the CE/J variant is a hybrid molecule sharing structural features with both SAA_1 and SAA_2 (11). All of these SAAs are dramatically induced by cytokines and can become major apolipoproteins on HDL. Of the acute phase mouse SAAs, only SAA_2 is rapidly cleared and deposited into amyloid fibrils (22, 23). Because of the differential clearance between SAA_1 and SAA_2 (23), we chose to overexpress the hybrid CE/J protein as a "generic" acute phase SAA. The use of adenoviral vectors to overexpress SAA allows for studying the effects of this apolipoprotein in the absence of other acute phase responses. A major finding of this study is that even though SAA can be the major apolipoprotein of HDL during severe acute phase states, it is not capable of substituting for apoA-I in the formation of HDL particles.

The recombinant adenoviruses used in these experiments produced high levels of expression of CE/J SAA and human apoA-I in mice. Given the similarity of the adenoviral vectors and the administered doses, the difference in the plasma levels of apoA-I (2.8 mg/ml) and SAA (760 μg/ml) could be due to the more rapid clearance (24) or relative extravascular distribution of SAA compared to apoA-I. The induction of endogenous SAAs could not be detected, indicating that a general acute phase response was not induced by the adenoviral treatments. Injection of Ad-apoAI resulted in a significant increase in HDL cholesterol, to a level exceeding that of normal mice. A large portion (approximately 62%) of plasma apoA-I could be recovered in the high density fraction by flotation, indicating that the human apoA-I reconstituted HDL particles in apoA-I (−/−) mice. In contrast, overexpression of SAA resulted in only a modest increase in HDL cholesterol levels, and only a very small portion (4%) of SAA could be recovered in the HDL fraction by density centrifugation. FPLC analysis revealed that the bulk of SAA does not chromatograph with cholesterol. Analysis of plasma from Ad-SAA-treated apoA-I (−/−) mice by non-denaturing acrylamide gel electrophoresis failed to define a particle of discrete size. Sudan black staining indicated a diffuse smear considerably larger than HDL (data not shown). It is thus likely that the 2-fold increase in HDL cholesterol produced by Ad-SAA is due to an increase in the diameter of HDL particles. These data indicate that the bulk of SAA in the apoA-I (−/−) mice is not associated with HDL as conventionally defined. It has previously been shown that SAA aggregates in aqueous solutions (24). Our data suggest that the plasma of Ad-SAA-treated mice contains mostly aggregates of SAA. These aggregates, which cannot be floated at the density of HDL, comprise the bulk of plasma SAA in these mice. The small amount of SAA that can be floated therefore seems to be an "add on" to the particles that already exist in the apoA-I (−/−) mice.

A feature of the HDL recovered from the Ad-SAA-

treated apoA-I (−/−) mice was the unusually high unesterified cholesterol component. A decrease in esterified cholesterol and LCAT activity in plasma of apoA-I (−/−) mice has been demonstrated previously (25). In the apoA-I (−/−) mice this decrease in LCAT activity resulted, in part, from a decrease in HDL substrate particles and, in part, from the absence of apoA-I, the major co-factor for LCAT. Introduction of SAA by Ad-SAA in our studies further reduced LCAT activity, indicating that SAA in the absence of apoA-I does not generate HDL particles capable of acting as substrates for LCAT-mediated cholesterol esterification. This is consistent with the report that high plasma levels of SAA are inversely related to plasma cholesteryl ester concentrations and LCAT activity, and that high levels of SAA in phospholipid vesicles decreased LCAT function in vitro (26).

Our results confirmed the observation that apoE concentrations in plasma are increased in the absence of apoA-I (10). High level expression of SAA resulted in a further 2-fold increase in plasma apoE levels in the apoA-I (−/−) mice. This increase in plasma apoE was accompanied by an increase in the amount of apoE associated with the HDL fraction. Ad-SAA treatment also resulted in an increase in the amount of apoA-IV associating with HDL particles. However, in contrast to apoE, apoA-IV plasma levels were not altered by Ad-SAA treatment. It is possible that more plasma apoA-IV is recruited onto a subset of particles that is increased in the Ad-SAA-treated mice. The HDL particles produced in the Ad-SAA-treated mice were not homogeneous, as apoE generally eluted with larger particles and SAA and apoA-IV eluted with smaller particles. These data reveal an intricate complexity in the way in which apolipoproteins and lipids associate to form lipoprotein particles.

The presence of significant amounts of SAA in non-HDL-associated aggregates may have implications for amyloidogenesis. It is conceivable that if SAA is produced in excess (as a result of inflammation) and cannot form HDL, this non-particle-associated SAA could be more rapidly cleared from the plasma and deposited in tissues.

The data indicate that HDL particles isolated from mice and humans containing SAA as the predominant lipoprotein are likely the result of metabolic processes in which apoA-I plays an essential role. Unlike apoA-I, SAA appears incapable of recruiting phospholipid to form discrete lipoprotein particles. This conclusion is in agreement with previous immunoaffinity data indicating that acute phase human plasma does not contain particles comprised of only SAA.(3) We conclude that even though SAA can associate avidly with normal HDL_3 particles and displace apoA-I (3), it seems incapable of forming such particles de novo.

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