Role of Serum Amyloid A During Metabolism of Acute-Phase HDL by Macrophages

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Abstract—The serum amyloid A (SAA) family of proteins is encoded by multiple genes that display allelic variation and a high degree of homology in mammals. Triggered by inflammation after stimulation of hepatocytes by lymphokine-mediated processes, the concentrations of SAA may increase during the acute-phase reaction to levels 1000-fold greater than those found in the noninflammatory state. In addition to its role as an acute-phase reactant, SAA (104 amino acids, 12 kDa) is considered to be the precursor protein of secondary reactive amyloidosis, in which the N-terminal portion is incorporated into the bulk of amyloid fibrils. However, the association with lipoproteins of the high-density range and subsequent modulation of the metabolic properties of its physiological carrier appear to be the principal role of SAA. Because SAA may displace apolipoprotein A-I, the major protein component of native high density lipoprotein (HDL), during the acute-phase reaction, the present study was aimed at (1) investigating binding properties of native and acute-phase (SAA-enriched) HDL by J774 macrophages, (2) elucidating whether the presence of SAA on HDL particles affects selective uptake of HDL-associated cholesteryl esters, and (3) comparing cellular cholesterol efflux mediated by native and acute-phase HDL. Both the total and the specific binding at 4°C of rabbit acute-phase HDL were 2-fold higher than for native HDL. Nonlinear regression analysis revealed $K_d$ values of $7.0 \times 10^{-7}$ mol/L (native HDL) and $3.1 \times 10^{-7}$ mol/L (acute-phase HDL), respectively. The corresponding $B_{\text{max}}$ values were 203 ng of total lipoprotein per milligram of cell protein (native HDL) and 250 ng of total lipoprotein per milligram of cell protein (acute-phase HDL). At 37°C, holoparticle turnover was slightly enhanced for acute-phase HDL, a fact reflected by 2-fold higher degradation rates. In contrast, the presence of SAA on HDL specifically increased (1.7-fold) the selective uptake of HDL cholesteryl esters from acute-phase HDL by J774 macrophages, a widely used in vitro model to study foam cell formation and cholesterol efflux properties. Although ligand blotting experiments with solubilized J774 membrane proteins failed to identify the scavenger receptor-BI as a binding protein for both native and acute-phase HDL, 2 binding proteins with molecular masses of 100 and 72 kDa, the latter comigrating with CD55 (also termed decay-accelerating factor), were identified. During cholesterol efflux studies, it became apparent that the ability of acute-phase HDL with regard to cellular cholesterol removal was considerably lower than that for native HDL. This was reflected by a 1.7-fold increase in $T_1/2$ values (22 versus 36 hours; native versus acute-phase HDL). Our observations of increased HDL cholesteryl ester uptake and reduced cellular cholesterol efflux (acute-phase versus native HDL) suggest that displacement of apolipoprotein A-I by SAA results in considerably altered metabolic properties of its main physiological carrier. These changes in the apolipoprotein moieties appear (at least in the in vitro system tested) to transform an originally antiatherogenic into a proatherogenic lipoprotein particle. (Arterioscler Thromb Vasc Biol. 2000;20:763-772.)

Key Words: inflammation ■ cholesterol metabolism ■ HDL

Serum amyloid A (SAA) is a generic term for a family of apolipoproteins coded for by different genes with high allelic variation and a high degree of homology between species. The SAA proteins in mammals (12 kDa protein, 104 amino acids) are very well conserved throughout evolution, and thus a panel of different activities and biological properties has been suggested. SAA may suppress lymphocytic response to antigens, impair platelet aggregability, and contribute to the regulation of tissue collagenase expression. SAA induces mast cell adhesion to the extracellular matrix, migration and adhesion of T cells, and migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. SAA also induces calcium mobilization and chemotaxis in monocytes and enhances eicosanoid synthesis in human monocytes. However, 3 major functions of SAA must be considered.

First, SAA is predominantly produced by the liver. The synthesis is largely regulated by inflammation-associated cytokines, peptide hormone signals produced by endothelial cells, lymphocytes, and in particular, activated monocytes.
and macrophages. Different cytokines, including interferon-γ, transforming growth factor-β, tumor necrosis factor-α, and interleukins, either alone or in combination, have been shown to affect SAA synthesis at the transcriptional level. This effect is mediated via cis-acting promoter elements that are binding sites for cytokine-activated nuclear factors, acute-phase protein factor, and the glucocorticoid receptor and liver specific transcription factors. In the circulation, SAA levels may increase by 1000-fold in response to injury, infection, and inflammation, and thus SAA has properties resembling a classic positive acute-phase reactant. Because concentrations of acute-phase reactants may be correlated with the amount of damaged tissue, measurements of SAA are of value in the assessment of activity and response to therapy during several inflammatory diseases.

Second, chronically elevated concentrations of acute-phase SAA are a prerequisite for the pathogenesis of secondary amyloidosis, a progressive and fatal disease characterized by the deposition in major organs of insoluble plaque composed principally of proteolytically cleaved acute-phase SAA, and may also contribute to processes that lead to atherosclerosis. SAA is deposited in the spleen, kidney, and liver, where it is processed, leaving the amino half to two thirds of its sequence behind in the fibril. How SAA becomes deposited as an amyloid A protein (the 76–amino acid N-terminal portion of SAA) is largely unknown; however, it is possible that its physiological carrier (see below) could contribute to this process.

Finally, as shown first in mice but later also in other mammals, SAA associates rapidly during the acute phase with HDL, on which it becomes the predominant apolipoprotein (apo SAA), exceeding apo A-I (the major apolipoprotein of native HDL) in quantity. Although significant amounts of apo SAA were also found to be associated with other lipoproteins, it seems that HDL subfraction 3 (HDL₃) is the preferential acceptor particle. SAA-enriched HDL₃ was found to be larger than normal HDL₃ (d=1.12 to 1.21 g/mL), having a radius of 4.5 to 5.3 nm that extended into the size range observed for normal HDL₂ (d=1.063 to 1.125 g/mL). SAA may account for 17% to 87% of the total apolipoproteins present in acute-phase HDL, and as a consequence, the percentage of lipid-free apo A-I may increase in parallel. While it remodels the lipoprotein particle by displacing primarily apo A-I, apo SAA may also significantly alter the protective function of its physiological carrier. Impaired activities of HDL-associated enzymes, ie, paraoxonase and the platelet-activating factor acetylhydrolase, are probably responsible for the impaired antioxidative properties of HDL during the acute phase. Subsequently, impaired activity of lectin:cholesterol acyltransferase, an enzyme activated by apo A-I, might be responsible for altered cholesterol hemostasis of acute-phase HDL compared with native HDL. Acton et al have shown that scavenger receptor-BI (SR-BI), a CD36-related class B type 1 scavenger receptor, is involved in selective uptake of HDL lipids and that apo A-I may mediate SR-BI–dependent hepatic selective uptake of HDL-associated cholesteryl esters (HDL-CEs). During this process, HDL-CEs are taken up in vivo or in vitro without concomitant lipoprotein particle uptake. On the basis of these findings, altered binding properties of acute-phase HDL (as a result of apo A-I displacement) to hepatocytes compared with native HDL could be anticipated. Finally, the participation of native HDL in “reverse cholesterol transport” from peripheral cells to the liver is critical for the antiatherogenic properties of this lipoprotein. Because the efflux of cholesterol from peripheral tissues is mediated by HDL and (at least in part) via SR-BI, changes in the apolipoprotein moiety during the acute-phase reaction are tightly coupled with altered cholesterol homeostasis. It may be assumed that acute-phase HDL could mediate phospholipid and cholesterol CE delivery to regenerating tissue at sites of inflammation, where neutrophils, monocytes, and macrophages are present. This concept has been supported by the observation that acute-phase HDL binds strongly to peritoneal macrophages. Alternatively, it was hypothesized that acute-phase SAA targets the HDL particle to activated macrophages, where it enhances clearance of excess cholesterol from these cells.

The purpose of the present study was 2-fold: first, to determine binding properties, including association, internalization, and degradation, of native and acute-phase (SAA-enriched) HDL to macrophages; and second, to elucidate whether selective uptake of HDL-CEs is increased by the presence of SAA on acute-phase HDL particles and whether reverse cholesterol transport from these cells is altered in parallel. Because a number of studies have indicated that cholesterol and lipoprotein changes (including changes in HDL-associated apolipoproteins) during the acute-phase reaction in rabbits closely resemble those seen in humans, we used rabbit lipoproteins during our experiments. In addition, enrichment of rabbit HDL by SAA under inflammatory conditions in vivo is similar to that in humans, and finally, owing to the high homology of rabbit and human SAA isoforms, identical surface-located SAA epitopes present on acute-phase HDL particles in rabbits and humans can be anticipated.

Methods

Rabbit Lipoproteins

Rabbit plasma was obtained from New Zealand White rabbits (weight, 3 to 4 kg each), which were obtained from Charles River Germany (Sulzfeld). The acute phase was induced by injection of a 1% croton oil (vol/vol) emulsion in saline (1 mL/kg) into 5 sites in the larger lower-back muscle. Each rabbit served as its own control. Blood samples were drawn from the central ear artery into tubes containing 0.1% EDTA, pH 7.4, before and 48 hours after the injection of croton oil. HDL (d=1.063 to 1.21 g/mL) was prepared by discontinuous density ultracentrifugation of plasma. HDL was recovered from the tubes, dialyzed against PBS (10 mmol/L, pH 7.4, 0.15 mol/L NaCl), and stored at 4°C in the presence of EDTA and NaN₃. Protein concentrations of the lipoprotein particles were determined by the Lowry procedure with BSA used as the standard. Individual apolipoprotein content was established by pyridine extraction of Coomassie blue–stained bands from SDS–polyacrylamide gel electrophoresis (PAGE) on 10% to 20% gels and subsequent densitometric evaluation. Concentrations of phospholipids (bioMerieux), total cholesterol (Greiner), free cholesterol (Merck), and triglycerides (bioMerieux) were assayed with commercially available enzymatic kits.

Lipoprotein Labeling

Labeling of the Protein Moiety

Iodination of rabbit HDL particles was performed as described by Sinn et al with N-Br-succinimide as the coupling agent. Routinely,
Lipid-associated activity was always specific. This procedure resulted in specific activities between 8 to 15 counts per minute per nanogram protein. Non-specific lipid-associated activity was always <3% of total activity.

**Labeling of the Lipid Moiety**

Native and acute-phase rabbit HDLs were labeled with [1,2,6,7-3H]-cholesterol (DuPont NEN) by CE transfer protein–catalyzed transfer from donor liposomes as described previously. In brief, 200 μCi of the corresponding label and 100 μg of egg yolk lecithin (Sigma) were dried under argon, followed by the addition of 1 mL of PBS. The mixture was shaken for 2 minutes at 37°C and sonicated. Lipoproteins (1 mL, containing 3 to 6 mg of protein), 1 mL of rabbit lipoprotein-deficient serum (LPDS, as a source of CE transfer protein), and 1 mL of PBS were added. The mixture was incubated under argon at 37°C in a shaking water bath overnight. Subsequently, the labeled HDL fractions were reisolated in a TLX120 bench-top ultracentrifuge in a TLA100.4 rotor (Beckman) as described previously. The HDL band was aspirated and dialyzed against 10 mmol/L PBS, pH 7.4. This labeling procedure resulted in specific activities of 8 to 15 counts per minute per nanogram protein.

**Cell Culture Studies**

Permanent mouse J774 macrophages were plated on 6- or 12-well plates (Costar) in RPMI-1640 medium (Bio Whitaker, containing 10% [vol/vol] fetal calf serum [FCS] and L-glutamine) under standard conditions (37°C, 5% CO2, 95% humidity). Twelve hours before the experiments, the cells were incubated in RPMI-1640 containing 10% (vol/vol) LPDS as indicated (concentrations of free apo A-I were 3.5 μg/mL medium containing 10% LPDS).

**Binding Studies**

Binding studies of native and SAA-enriched (acute-phase) rabbit HDLs to J774 macrophages were performed at 4°C with increasing amounts of [3H]-labeled lipoproteins in the absence (total binding) or presence of a 20-fold excess (nonspecific binding) of unlabeled autologous lipoprotein species. Iodinated HDL particles were added to a final concentration of 50 μg protein/well, and cells were incubated in RPMI-1640 containing 10% (vol/vol) LPDS at 4°C for 5 hours. After this incubation, the medium was aspirated, and the cells were washed twice in Tri-buffered saline (TBS) containing 5% (vol/wt) BSA followed by 2 washes in PBS. Cells were lysed with 0.3N NaOH (1 mL, 1 hour at 4°C) to determine bound radioactivity and cell protein in the lysate. Protein measurement was performed as described previously. Specific binding (4°C) was calculated as the difference between total and nonspecific binding.

To determine bound, internalized, and degraded lipoproteins, cells were incubated at 37°C for 5 hours as described above with the same amounts of labeled and unlabeled lipoproteins. Subsequently, the medium was aspirated, and the cells were washed as described above. To release cell membrane–bound HDL particles, the cells were incubated at 4°C for 1 hour in the presence of trypsin (0.05%, Cytosystems). The trypsin-releasable fraction is referred to as “bound” fraction. Cells were then lysed in 0.3N NaOH to determine both the non–trypsin-releasable fraction (“internalized” fraction) and the cell protein in the lysate. We estimated degradation of native and acute-phase iodinated HDL particles by J774 macrophages by measuring the non–trichloroacetic acid (TCA)-precipitable radioactivity in the medium after precipitation of free iodine with AgNO3. In brief, 0.5 mL of medium was removed, mixed with 100 μL of BSA (30 mg/mL) and 1 mL of TCA (3 mol/L, 4°C), and left at 4°C for 30 minutes. Subsequently, 250 μL of AgNO3 (0.7 mol/L) was added and mixed, and the samples were centrifuged at 3000 rpm at 4°C for 15 minutes. One milliliter of the supernatant was counted on a gamma counter.

To determine holoparticle and selective uptake of HDL-associated CEs during the same experiment, the cells were incubated with [3H]-cholesterol (16:0)–labeled native and acute-phase HDL at 37°C for 5 hours. After removing the medium and washing the cells, we estimated cell association by measuring the radioactivity and protein content of the cell lysates, respectively. Specific binding/internalization/degradation/cell association was calculated as the difference between total and nonspecific binding/internalization/degradation/cell association.

To facilitate the comparison of results obtained with [3H]-labeled and [125I]-labeled lipoproteins, selective HDL and acute-phase HDL uptakes are expressed as apparent HDL particle uptake, as suggested by Pittman et al. Apparent HDL particle uptake is expressed in terms of HDL protein (calculated on the basis of the specific activity of the corresponding labeled HDL preparation used) that would be necessary to deliver the observed amount of tracer. These calculations were performed to compare uptake of [3H]- and [125I]-tracers on the same basis.

**Efflux Experiments**

Efflux of labeled cholesterol from J774 macrophages was measured by appearance of [3H]-cholesterol in the cellular supernatant and remaining radioactivity in cell lysates. The cells were incubated in the presence of RPMI-1640 containing 10% (vol/vol) LPDS and [3H]-cholesterol (0.05 μCi/mL) for 24 hours. Before the cholesterol efflux experiments were performed, the [3H]-cholesterol-containing medium was aspirated, and the cells were washed twice with TBS (containing 5% [wt/vol] BSA) and twice with TBS. Efflux experiments were initiated by the addition of native or acute-phase HDL (0.5 mg protein/mL) in RPMI-1640 containing 10% (vol/vol) LPDS. At the indicated time points (up to 24 hours), the medium was collected, and the cells were washed as described above. Then, the cells were lysed in 0.3N NaOH to estimate both the remaining radioactivity and the cellular protein content. Efflux of radioactive label to the medium was calculated as the percentage of radioactivity present in the cells before the addition of native or acute-phase HDL-containing medium.

**SDS-PAGE, Immunoblot, and Ligand-Blot Analyses**

SDS-PAGE of native and acute-phase rabbit HDL apolipoproteins was performed with 10% to 20% polyacrylamide gradient gels with electrophoresis at 150 V for 90 minutes in a Bio-Rad mini protein chamber and buffers as described previously. Samples for SDS-PAGE (2.5 to 50 μg protein) were treated with sample buffer (0.1 mol/L Tris/HCl, pH 6.8, 4% SDS, 15% glycerol, and 1% mercaptoethanol) at a ratio of 1:1 (vol/vol) and incubated at 95°C for 5 minutes before application to gels. J774 macrophages were cultured in RPMI-1640 (containing 10% FCS or LPDS), and cell membrane proteins were prepared according to Schneider et al. SDS-PAGE of membrane proteins (40 μg/lane) was performed with 8% polyacrylamide gels. For Western blotting experiments, proteins were electrophoretically transferred to nitrocellulose membranes (150 mA, 4°C, 90 minutes).

Immunochromatographic detection of proteins from solubilized membrane protein fractions was performed with the following primary antibodies: for identification of SR-BI, a polyclonal, sequence-specific rabbit anti-rat SR-BI peptide (496-509) antibody (dilution 1:100) was used; for identification of CD55, mouse anti-human CD55 antibody (dilution 1:200) was purchased from Serotec. Peroxidase-conjugated goat anti-rabbit IgG (diluted 1:2000) or rabbit anti-mouse IgG (diluted 1:2000) was used as a secondary antibody. To visualize immunoreactive bands, enhanced chemiluminescence (ECL)-Western blotting detection reagents and Cronex medical x-ray films (Sterling Diagnostic Imaging USA) were used according to the manufacturer’s suggestions.

For ligand blotting experiments, the nitrocellulose membranes were incubated with blocking buffer containing 50 μg of either native or acute-phase HDL per milliliter, and the blots were incubated at 4°C or 25°C on a rotating platform. After washing, the membranes were incubated with rabbit anti-apo A-I antisera (1:1000) or anti-human SAA antisera overnight, and visualization of bound lipoprotein was performed with peroxidase-conjugated goat antirabbit IgG (diluted 1:2000) as a secondary antibody after ECL development.

**Results**

**Characterization of Native and Acute-Phase Rabbit HDL Particles**

Before we studied the binding properties of native and acute-phase HDLs to J774 cells, the particles were character-
ized with respect to changes in the protein (Figure 1) and the lipid moiety (Table) due to the inflammatory response. Electrophoretic characterization of rabbit HDL particles revealed that after a 48-hour inflammation period, levels of apo A-I were decreased, whereas at the same time, levels of SAA were increased (Figure 1, lanes A and B). SAA levels ranged between 22% and 42% of total apolipoprotein content. A comparison of the lipid composition of HDL particles from the same rabbits before and 48 hours after induction of the acute phase revealed that the phospholipid content was also slightly increased during the acute phase, but a marked increase in triglyceride concentration was observed (Table 1); these data are in line with those of other reports.35,36 Similar changes in phospholipid and triglyceride concentrations became apparent in sera obtained from rabbits during the acute-phase reaction (Table 1).

**Binding of Native and SAA-Containing HDL Particles to Macrophages**

To investigate binding properties of native and SAA-containing HDL particles to J774 macrophages at 4°C, the protein moiety was labeled with [125 I]NaI. Binding of 125 I-labeled native and acute-phase rabbit HDLs in the presence or absence of a 20-fold excess of unlabeled lipoprotein is presented in Figure 2 (A and B). Both the total (119.1 versus 51.5 ng lipoprotein/mg cell protein) and the specific binding of rabbit acute-phase HDL (73.6 versus 37.5 ng lipoprotein/mg cell protein) were higher than for native HDL at the highest lipoprotein concentrations used. Binding experiments with 3 different preparations and subsequent calculation by nonlinear regression analysis (with the GraphPad program) yielded mean $K_d$ values of $7.0 \times 10^{-7}$ mol/L (native HDL) and $3.1 \times 10^{-7}$ mol/L (acute-phase HDL), respectively. The corresponding $B_{max}$ values were 203 ng total lipoprotein/mg cell protein (native HDL) and 250 ng total lipoprotein/mg cell protein (acute-phase HDL), assuming a molecular mass of $3 \times 10^5$ kDa and a protein content of $\approx 50\%$ (wt/wt). Similar binding properties were observed when rabbit HDL was isolated by discontinuous density ultracentrifugation at $d=1.125$ to 1.21 g/mL.

<p>| Protein Content and Lipid Composition of HDL and the Corresponding Serum Samples Obtained From Control (n=5) and Croton-Oil–Treated Rabbits (n=5) |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Native HDL</th>
<th>Acute-Phase HDL</th>
<th>Native Serum</th>
<th>Acute-Phase Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>199.38±6.11</td>
<td>201.30±13.94</td>
<td>5436±746</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>40.85±15.83</td>
<td>37.40±11.78</td>
<td>48.97±6.31</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>294.06±29.56</td>
<td>309.96±20.83</td>
<td>12.91±2.24</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>129.49±20.37</td>
<td>198.48±56.74</td>
<td>73.35±27.27</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>140.49±20.37</td>
<td>198.48±56.74</td>
<td>73.35±27.27</td>
</tr>
</tbody>
</table>

Values are given in $\mu$g/mg HDL protein (*) or mg/dL serum (?).
The next series of experiments was designed to study binding properties of $^{125}$I-labeled native and acute-phase rabbit HDL particles at 37°C in the presence or absence of a 20-fold excess of unlabeled lipoproteins. Bound lipoproteins were released with trypsin from the cells, and the trypsin-releasable fraction was referred to as bound fraction. Binding of native HDL to J774 macrophages was saturable and specific (Figure 3A). For acute-phase HDL, no plateau value was obtained under the same experimental conditions (Figure 3B). Specific binding was 41.5 ng/mg cell protein (native HDL) and 27.2 ng/mg cell protein (acute-phase HDL) at the highest protein concentrations used. Next, the radioactivity was measured in the non–trypsin-releasable fraction, which is referred to as the internalized fraction. Compared with the trypsin-releasable radioactivity, the internalized radioactivity was much higher for both lipoprotein particles (Figures 3C and 3D). Specific internalization revealed similar values for native (234.1 ng lipoprotein/mg cell protein) and acute-phase (242.5 ng lipoprotein/mg cell protein) HDL at the highest protein concentrations used. Next, the radioactivity was measured in the non–trypsin-releasable fraction, which is referred to as the internalized fraction. Compared with the trypsin-releasable radioactivity, the internalized radioactivity was much higher for both lipoprotein particles (Figures 3C and 3D). Specific internalization revealed similar values for native (234.1 ng lipoprotein/mg cell protein) and acute-phase (242.5 ng lipoprotein/mg cell protein) HDL at the highest protein concentrations used. In a parallel series of experiments, the degradation of $^{125}$I-labeled native and acute-phase HDL particles by J774 macrophages was saturable and specific (Figure 3A). For acute-phase HDL, no plateau value was obtained under the same experimental conditions (Figure 3B). Specific binding was 41.5 ng/mg cell protein (native HDL) and 27.2 ng/mg cell protein (acute-phase HDL) at the highest protein concentrations used. Next, the radioactivity was measured in the non–trypsin-releasable fraction, which is referred to as the internalized fraction. Compared with the trypsin-releasable radioactivity, the internalized radioactivity was much higher for both lipoprotein particles (Figures 3C and 3D). Specific internalization revealed similar values for native (234.1 ng lipoprotein/mg cell protein) and acute-phase (242.5 ng lipoprotein/mg cell protein) HDL at the highest protein concentrations used. In a parallel series of experiments, the degradation of $^{125}$I-labeled native and acute-phase HDL particles by J774 macrophages was estimated by measurement of the non–TCA-precipitable radioactivity in the medium after precipitation of free iodine with AgNO$_3$. From Figures 3E and 3F, it is evident that specific degradation of acute-phase HDL particles was $\approx$1.5-fold higher than for native HDL (104±27 versus 72±38 ng lipoprotein/mg cell protein) at the highest HDL concentrations used.

To determine the ability for selective uptake of HDL-associate CEs, J774 macrophages were incubated in the presence of $^{125}$I-labeled and $[^{3}H]$cholesterol (16:0)–labeled native and acute-phase HDLs. In the case of $^{125}$I-labeled HDL, the cell-associated and non–TCA-precipitable radioactivity in the medium was counted (sum of cell-associated and degraded HDL reflects holoparticle uptake). For the $[^{3}H]$cholesterol (16:0)–labeled HDL preparations, only cell-associated radioactivity was counted and calculated as apparent particle uptake. The difference between apparent particle uptake and holoparticle uptake reflects selective HDL-CE uptake.

As shown for iodinated HDL, specific cell association of $[^{3}H]$cholesterol (16:0)–labeled native HDL was also lower than $[^{3}H]$cholesterol (16:0)–labeled acute-phase HDL (868 versus 1337 ng lipoprotein/mg cell protein; Figures 4A and 4B). In line with reports for mouse peritoneal macrophages and permanent J774 macrophages, we have observed pronounced capacity for lipid tracer uptake in excess of particle association, exceeding holoparticle uptake by 2.6-fold for native HDL (868 versus 330 ng lipoprotein/mg cell protein) (Figure 5). Selective uptake of HDL-CE from native HDL particles exceeded holoparticle uptake by 1.6-fold (538 versus 330 ng lipoprotein/mg cell protein) at the highest HDL concentrations used (Figure 5A). Selective uptake of HDL-CE from acute-phase HDL exceeded holoparticle uptake by 2.1-fold (901 versus 435 ng lipoprotein/mg cell protein) at the highest HDL concentrations used (Figure 5B). However, total CE delivery (selective and holoparticle up-
CD55, also termed decay-accelerating factor (DAF), as a possible binding protein for native HDL. The 72-kDa HDL-binding protein identified in our ligand blots aligned with the 72-kDa protein recognized by anti-CD55 (Figure 6, lane 3), whereas the 110 kDa demonstrated an unspecific immunological cross-reactivity. Although a sequence-specific polyclonal antiserum (prepared against the 15 C-terminal amino acids of rat SR-BI) detected 2 proteins of ≈82 and 78 kDa (Figure 6, lane 4), in line with other reports, we could not detect HDL binding to J774 proteins compared with these proteins recognized by polyclonal anti–SR-BI peptide antiserum.

**Effect of Native and Acute-Phase HDLs on Cellular Cholesterol Efflux**

We have been interested in whether the in vivo exchange of apo A-I by apo SAA may alter the efficiency of HDL to promote cholesterol efflux from J774 macrophages, a widely used in vitro model to study foam cell formation and cholesterol efflux properties. Results of a representative experiment are shown in Figure 7. During incubation with the radioactive tracer, cells acquired 44.7 ± 4.3 dpm/mg of cell protein (n = 3; 33% of the initial radioactivity added). After a 24-hour incubation of macrophages in the presence of native HDL, the cellular cholesterol content decreased to 43.8 ± 3.0%. However, 58.9 ± 2.9% of the cholesterol remained cell-associated when the efflux experiment was performed in the presence of acute-phase (SAA-enriched) HDL. Accordingly, the radioactivity present in the medium (after a 24-hour incubation) was 50.8 ± 1.6% and 38.0 ± 1.9% of the initial radioactivity when cells were cultivated in the presence of native and acute-phase HDLs, respectively. The lower ability of acute-phase HDL to promote cellular cholesterol efflux was also reflected by a significant, 1.6-fold increase in n/τ (time necessary to remove 50% of cholesterol; 22 versus 36 hours, native versus acute-phase HDL). Similar results were obtained when SAA enrichment of acute-phase rabbit HDL particles was 22% only or increased to 37% (graph not shown).

**Discussion**

There is ample evidence that acute inflammation interferes with the metabolism of lipids and lipoproteins and affects the concentration and composition of different lipoproteins and/or associated enzymes involved. In mammals, plasma concentrations of apolipoproteins, in particular apo A-I, decrease significantly, but levels of triglyceride-, phospholipid-, and cholesterol-rich particles are also changed in parallel. To study acute inflammation in animals, 2 types of injury are generally used: localized inflammation induced by the formation of a sterile abscess after subcutaneous injection of oils and systemic inflammation induced by intravenous injection of lipopolysaccharide. Although slight interspecies differences in the inflammatory response may occur between both types of inflammation, rabbits have been proven as a suitable animal model to study inflammation-related changes in lipoprotein and apolipoprotein metabolism, therefore, we decided to use rabbit acute-phase HDL as a source of SAA-containing particles. The enrichment of rabbit HDL with SAA during the acute-phase reaction occurs in a similar manner as described for...
humans, and the acute-phase SAA isoforms of the 2 species are highly homologous. The overall percentage identity between human and rabbit SAA is 83.3% (for SAA1, the main SAA isoform, which constitutes >90% of total SAA expressed during the acute-phase reaction) and 80.4% (for SAA2).2 Surface-located epitopes of HDL-associated SAA (which are most likely responsible for acute-phase HDL-cell interactions) revealed identities of 87.5% (amino acids 31 to 39), 89% (amino acids 64 to 78), and 80% (amino acids 95 to 104) for human and rabbit SAA1, respectively.37,38 Finally, epitopes of human apo A-I assumed to be responsible for binding and cholesterol efflux properties from cells60–62 have an overall identity and similarity with rabbit apo A-I of 81% and 89.5%, respectively. Therefore, rabbit acute-phase HDL serves a suitable model to mimic inflammation-related lipoprotein changes occurring in humans and represents an experimentally easily accessible source of SAA-containing lipoproteins and corresponding autologous controls.

The major findings of the present study can be summarized as follows: (1) increased binding of acute-phase HDL at 4°C; (2) slightly higher holoparticle turnover at 37°C, reflected by higher degradation rates of acute-phase HDL; (3) acute-phase and native HDLs bind to the same J774 proteins on nitrocellulose; and (4) compared with native HDL, acute-phase HDL has a lower capacity to promote cellular cholesterol efflux. Our observations concerning the increased binding affinity of SAA-containing HDL particles are in line with a previous report performed with mouse peritoneal macrophages.33 During the present study, we observed differences in binding properties between native and acute-phase HDL particles, suggesting that alterations in the protein moiety and lipid composition are the cause of the altered binding capacity of SAA-containing particles at 4°C. Also, at 37°C, holoparticle turnover was slightly higher for acute-phase HDL than for the native lipoprotein. Under the conditions used during the present study, this was best reflected by increased degradation rates of acute-phase HDL (~2-fold higher than for native HDL). As a consequence, total and selective CE uptake by J774 macrophages was higher for SAA-containing HDL. There is also clear evidence from in vitro and in vivo studies that scavenger receptors of the B class are responsible for selective uptake of HDL-associated CE, mediating reverse cholesterol transport and fuelling steroidogenesis. In line with other reports28,63 we could detect SR-BI in detergent-solubilized membrane protein fractions obtained from J774 cells; we were, however, unable to detect SR-BI–mediated HDL binding during ligand blotting experiments. The reason for this negative finding is presently not clear but might be a result of SR-BI inactivation due to conformational changes during SDS-PAGE and/or transfer to nitrocellulose. Although
we could not detect binding to a band comigrating with those recognized by anti–SR-BI peptide antiserum, we detected 2 proteins with molecular masses of 72 and 100 kDa that have bound native and acute-phase HDL on ligand blots. The 72-kDa protein colocalized with 1 protein recognized by anti-CD55 (anti-DAF). DAF is a GPI-anchored protein that protects cells from damage by autologous complement activation. Although our findings do not necessarily prove a role for DAF as an HDL binding protein on macrophages, reports of CD55 binding by LDL64 and HDL,65 localization of CD55 in caveolae,66 and expression on the surface of circulating blood cells, including monocytes,67 makes this hypothesis attractive. In addition, Nion et al34 recently suggested that DAF could act as a binding protein for native HDL on caveola-rich SMKES cells. Whether the 100-kDa band is identical to HBs,53 an HDL binding protein present on macrophages, remains to be established. Whether and to what extent endogenously expressed lipoprotein lipase could contribute to increased selective uptake of HDL-associated CEs31,68 is also unclear presently.

Recently, recombinant apo SAAα, a hybrid of human apo SAA1 and apo SAA2 isoforms, was shown to bind cholesterol with high affinity69 and to modulate uptake of free cholesterol by HepG2 cells. In human monocytic THP-1 cells, apo A-I is the principal mediator of cholesterol efflux,70 and therefore the presence of SAA on HDL had little impact on cellular cholesterol efflux except when apo SAA represented >50% of total HDL apoproteins.71 Our studies were performed with J774 mouse macrophages and have clearly demonstrated diminished capacity for cholesterol efflux by acute-phase HDL even at an SAA enrichment of only 22%. In particular, these SAA concentrations occur in a broad range of various physiologically occurring inflammatory events related to altered lipoprotein profiles.

SR-BI plays a key role during hepatic HDL metabolism in determining plasma HDL cholesterol levels and possibly by mediating cellular cholesterol efflux from peripheral cells. In line with a role of SR-BI during reverse cholesterol transport, a linear relationship between expression levels of SR-BI on different cell lines, including J774 cells, and the degree of HDL-mediated cellular cholesterol efflux was determined.28,63 Our findings of increased binding of acute-phase HDL and significantly reduced cellular cholesterol efflux from the cells do not exclude a possible role of SR-BI; as reported by de la Llera-Moya and colleagues.30 SR-BI–dependent stimulation of cholesterol efflux is not simply due to SR-BI–mediated HDL binding to the cell surface. These authors30 suggested that SR-BI expression could involve a generalized redistribution of membrane cholesterol to caveolae, thus facilitating cholesterol exchange to HDL. Phospholipid subspecies are an important determinant (in addition to others) in mediating cellular cholesterol efflux to a given acceptor particle. This, in conjunction with reports that HDL undergoes severe redistribution of phospholipid subspecies during the acute phase,19,33,36,57 could provide another plausible explanation for our findings relating to the reduced capacity of SAA-containing HDL for cellular cholesterol removal.

Although the association of SAA with HDL has been confirmed for a number of years, the physiological significance of this change remains controversial.31 Kisilevsky et al14 postulated that the principal role for SAA in acute...
inflammation is to enhance cholesterol removal from sites of tissue destruction, whereas Gonnermann et al. proposed that SAA may commandeer HDL during the acute-phase response to deliver phospholipids and cholesterol to cells involved in tissue repair at sites of inflammation. Our present in vitro data, in particular a 2-fold higher selective SAA-enriched HDL-C uptake by macrophages, provide support for the latter hypothesis. Increased selective uptake of acute-phase HDL CEs and subsequent hydrolysis could transform monocyte-derived macrophages into cholesterol-enriched foam cells, which are the hallmark of fatty streaks and the earliest recognizable lesion of atherosclerosis. The possibility that SAA could direct lipids to and modulate lipid flow in atherosclerotic lesions is further strengthened by the fact that SAA has been found to be expressed in human arterial walls that shows signs of atherosclerotic lesions. Members of the acute-phase SAA family are among the inflammatory genes that have also been found to be abundantly expressed in high-fat diet–enriched, atherosclerosis-susceptible C57BL/6 that have also been found to be abundantly expressed in MRL/lpr mice, whereas atherosclerosis-resistant C3H/HeJ mice did not express SAA at all. A similar phenomenon was observed in MRL/lpr mice fed an atherogenic diet. The presence of acute-phase SAA in atherosclerotic lesions, the induction of acute-phase SAA genes in human monocyte/macrophage cells mediated by minimally modified LDL, and the proinflammatory role of acute-phase HDL, in addition to enhanced selective uptake of HDL-associated CE as shown in the present study, hold implications for the involvement of SAA during atherogenesis.

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