Dynamic and stable pools of apoE differ functionally at the HepG2 cell surface

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Abstract **Synthesis of apolipoprotein (apo)E in hepatocytes leads to both secretion and retention in cell surface pools. Inclusion of Brefeldin A to HepG2 cells prompted a rapid decrease of cell surface apoE to about 37% of control values after a 3-h incubation. The $t_{1/2}$ for this dynamic pool was estimated to be 15 min. In contrast, a stable fraction of apoE ($t_{1/2} > 20$ h) was found in association with the extracellular matrix (ECM). Increased content of apoE on the ECM correlated with decreased binding of VLDL. Decreased apoE on the cell surface correlated with increased binding of VLDL to cells. Collectively, this suggests that glycosaminoglycan-bound apoE can occlude binding sites for apoE-containing lipoproteins on glycosaminoglycans. In solid-phase assays, heparin, suramin, and chondroitin sulfates A and B efficiently inhibited the binding of apoE to heparan sulfate proteoglycans, but were unable to displace apoE from this glycosaminoglycan. Finally, decreasing cell surface apoE with suramin subsequently decreased the apoE content on secreted apoB-containing lipoproteins without affecting the overall secretion of apoE or apoB to the extracellular medium. In summary, cell surface apoE comprises both dynamic fractions, which can be donated to newly secreted lipoproteins, and stable fractions, which may act to minimize the unproductive binding of lipoproteins to the ECM.**—Burgess, J. W., and Y. L. Marcel. **Dynamic and stable pools of apoE differ functionally at the HepG2 cell surface.** ***J. Lipid Res.*** **2001.** 42: **1413–1420.**

Supplementary key words apolipoprotein E • extracellular matrix • secretion • glycosaminoglycans

Apolipoprotein (apo)E is a component of plasma lipoproteins, and plays an important role in the transport of lipids in plasma, both as an exchangeable apolipoprotein and as a ligand for cell surface proteoglycans and receptors of the LDL receptor family (1, 2). The secretion of apoE has been studied in detail, particularly in hepatocytes and macrophages, which are major contributors of circulating apoE (3–6). However, the secretory pathway of newly synthesized apoE is complex and only partly characterized at present. In hepatocytes, newly synthesized apoE appears to be restricted to saccular distensions of the Golgi complex in contrast to albumin, which is homogeneously distributed throughout the Golgi (7). This segregation of apoE is analogous to that observed for newly synthesized heparan sulfate proteoglycan (HSPG); however, it was observed that apoE and albumin sorted into denser trans-Golgi network secretory vesicles than did HSPG (8). This suggests that during the last phase of its transit through the Golgi, apoE is not bound to HSPG. Regardless, about a third of newly synthesized apoE is delivered to the extracellular medium in HepG2 cells where it is found in association with lipoproteins (9, 10). It has been reported that conditions promoting hepatic lipogenesis markedly increase the secretion of apoB-containing lipoproteins, but has little effect on the secretion of apoE (11). This has led to the suggestion that the secretion of apoE occurs independently of apoB. It has also been proposed that the association of apoE with lipoproteins containing apoB occurs intracellularly (12).

A cell surface pool of apoE has been described on hepatocytes (13–15) and hepatoma cells (16–20). ApoE of the HepG2 cell surface accounts for as much as 54% of the total cellular apoE (20). Earlier evidence based on the release of cell surface apoE upon treatment with heparin and heparinase suggested attachment through HSPG (18, 19). However, we reported a heterogeneous association of apoE with the HepG2 cell surface. In these cells, apoE may be found in association with the extracellular matrix (ECM) or plasma membrane, be lipid-poor or lipidated, and associate through chondroitin sulfate proteoglycan (CSPG) or HSPG (16, 17). Multiple pools of cell surface apoE including dynamic and stable fractions, as well as high and low molecular weight forms that represent different extents of glycosylation, have also been described in J774 macrophage transfected with human apoE3 (21). Considerable evidence has accumulated indicating that cell surface apoE is important for chylomicron remnant metabolism. The apoE enrichment of lipoproteins en-

Abbreviations: apoB, apolipoprotein B; apoE, apolipoprotein E; CSPG, chondroitin sulfate proteoglycan; ECM, extracellular matrix; EMEM, Eagle's minimum essential medium; HSPG, heparan sulfate proteoglycan; LRP, LDL receptor-related protein; RAP, receptor-associated protein.

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hances their ability to bind to HSPG and lipoprotein receptors of the hepatocyte cell surface (secretion-capture model). The studies of Linton et al. (22) suggest that apoE of hepatic origin, but not free in the Space of Disse, is important for remnant metabolism in mice lacking a functional LDL receptor. Furthermore, bolus injections of remnants into transgenic mice expressing apoE significantly reduced the amount of apoE on the hepatocyte cell surface with concomitant increases in apoE associated with intracellular vesicles (15). More recently, it was observed that incubating HepG2 cells with exogenous lipoproteins decreases the levels of cell surface apoE (20). Although transfer of apoE from the cell surface to exogenous lipoproteins was not demonstrated in this study, direct transfer could provide an alternative mechanism for the enrichment of chylomicron remnants or newly secreted lipoproteins with apoE.

In the current study, we further characterized the association of apoE with the HepG2 cell surface, and attempted to ascribe functions to these multiple cell surface pools. The stable fraction of the ECM may prevent the unproductive binding of apoE-enriched remnants to glycosaminoglycans of the ECM; the rapidly turning-over pool can be transferred to extracellular lipoproteins, and may therefore function in the secretion-capture of apoE, allowing hepatocyte uptake of remnants, and in enriching newly secreted lipoproteins with apoE. We also provide evidence that heparin and suramin decrease cell surface apoE by inhibiting the binding of apoE from the medium to surface glycosaminoglycans rather than by effecting the displacement of apoE from glycosaminoglycans.

EXPERIMENTAL PROCEDURES

Materials

$Na^{125}I$ was obtained from Amersham (Oakville, ON). Iodobeads were from Pierce (Rockford, IL). Heparin from porcine intestinal mucosa, suramin, chondroitin sulfates A, B, and C, and purified HSPG were obtained from Sigma (St. Louis, MO). Tissue culture reagents were obtained from Sigma and GIBCO (Burlington, ON). HepG2 cells were obtained from the ATCC.

Antibodies

Monoclonal antibody 7C9,[2] which recognizes residues 1–15 of the N-terminus of apoE, has been described (16, 17, 23–25). Iodination of 7C9 was performed by the Iodobead method (Pierce) and specific activities between 8.0–10.6 μCi/μg were obtained. Other monoclonal antibodies against apoE (3H1) and apoB (1D1) have been described (16, 17).

Cell culture and characterization of cell surface apoE

Culture of HepG2 cells and determination of cell surface apoE were performed as described (16, 17). Experiments were performed on day 4 after seeding. ECM was prepared by the Triton X-100 method or the cytochalasin B method (16, 26). The results for antibody binding are expressed as the mean specific binding/mg cell protein for cell monolayers or as mean specific binding/well for ECM.

ApoE binding to immobilized glycosaminoglycans

Purified HSPG from Engelbroth-Holm-Swarm mouse sarcoma was immobilized by passive adsorption to 96-well microtiter plates (Immobilon 2; Dynatech Laboratories) for 16 h at 4°C. Unbound material was removed with three washes of PBS, and the wells were blocked with 250 μl of 3% BSA in PBS for 1 h at room temperature. To examine binding of apoE to the glycosaminoglycans, apoVLDL prepared from VLDL as described (17) was added to the wells (2.5 μg/100 μl) and incubated in the absence or presence of heparin (1 mg/ml), suramin (0.5 mg/ml), or chondroitin sulfates A, B, or C (1 mg/ml) for 2 h at 37°C. The wells were washed three times with PBS and incubated with ^{125}I-7C9 (100,000 cpm/well) for 2 h at 20°C. Finally, the wells were washed three times with PBS and counted. Displacement assays were conducted as follows: apoVLDL was pre-incubated with the immobilized glycosaminoglycans as described above; unbound material was removed with three washes with PBS; and Eagle's minimum essential medium (EMEM)-BSA containing heparin, suramin, or chondroitin sulfates A, B, or C was added and incubated at 37°C for 1 h. The apoE associated with the wells was determined as described above.

Determination of the apoE content of secreted apoB-containing lipoprotein

1D1 in PBS (1 μg/well) was adsorbed to microtiter wells (Immobilon 2) for 16 h at 4°C. The wells were washed three times with PBS and blocked at 20°C with PBS containing 1% BSA for 30 min. Conditioned medium from control or suramin-treated HepG2 cells containing identical amounts of apoB as determined by radioimmunoassay was added (100 μl/well) and incubated for 2 h. The wells were washed three times with PBS, and ^{125}I-7C9 in PBS containing 2 mg/ml BSA was added and incubated with the adsorbed lipoproteins for 1 h at 20°C. The wells were washed three times with PBS and counted in a gamma counter.

Miscellaneous procedures

VLDL was prepared from the plasma of normolipidemic subjects by density gradient ultracentrifugation as described by Rall et al. (27). Media apolipoproteins were quantified by radioimmunoassay as previously described (17). Cell protein was determined by the method of Lowry et al. (28) with BSA as standard.

RESULTS

Heparin- and suramin-mediated release of cell surface apoE in cells treated with inhibitors of protein secretion

HepG2 cells were incubated at 37°C with EMEM-BSA containing heparin or suramin in the absence (**Fig. 1**, left and right panels) or presence of Brefeldin A (Fig.1, center panel). After washing at 4°C, the relative levels of cell surface apoE were determined by examining the specific binding of ^{125}I-7C9, a monoclonal antibody that recognizes residues 1–15 of the N-terminus of apoE, to the cell surface. In Fig. 1 (left panel), we show, in agreement with previous reports (17, 19), that incubations with heparin and suramin at 37°C reduce cell surface apoE by about 25% and 40%, respectively. Brefeldin A treatment alone reduced cell surface apoE by about 50% in these 1-h incu-

[2] 7C9, 3H1, and 1D1 are available from the Heart Institute Research Corporation at the University of Ottawa Heart Institute.

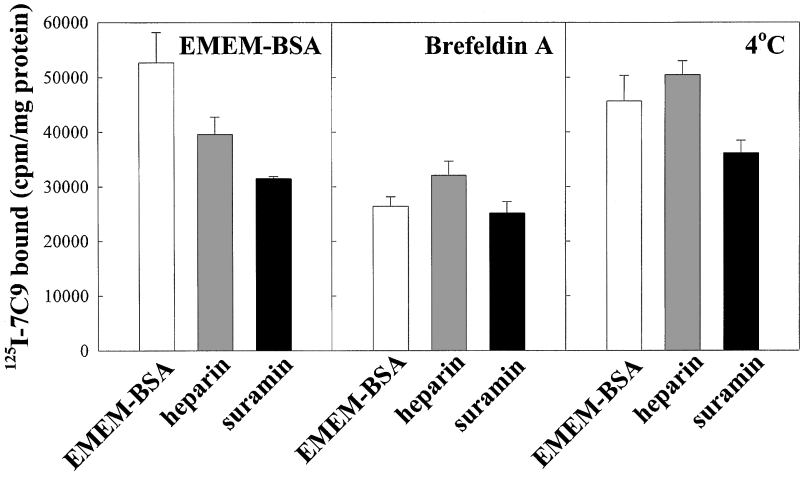

Fig. 1. Incubations with Brefeldin A or at 4°C remove the ability of heparin and suramin to reduce cell surface apoE. HepG2 cells were incubated for 1 h with EMEM-BSA containing heparin (1 mg/ml) or suramin (0.5 mg/ml) either in the absence (left and right panels) or presence of 2 μg/ml Brefeldin A (center panel). These initial incubations were done at 37°C (left and center panels) or at 4°C (right panel). Following these incubations, the cells were chilled on ice, washed twice with ice-cold EMEM-BSA, and the cell surface apoE content determined as described in Experimental Procedures. All data are the mean and SE of at least three separate determinations.

bations (Fig. 1, center panel). There was no further decrease in the levels of cell surface apoE with the inclusion of heparin (1 mg/ml) or suramin (0.5 mg/ml) to pre-incubations containing Brefeldin A (data not shown). The ability of heparin and suramin to reduce cell surface apoE was also eliminated when incubations were conducted at 4°C (Fig.1, right panel). This was observed with concentrations as high as 5 mg/ml of these reagents (data not shown).

One explanation for the observations with Brefeldin A or at 4°C is that a major portion of cell surface apoE is a dynamic pool that turns over rapidly and requires constant replacement from a secreted pool. To test this possibility, the levels of cell surface apoE were established as a function of time with Brefeldin A. The results of **Fig. 2** demonstrate that about 50% of cell surface apoE was lost rapidly within 1 h. The remaining pool was relatively stable and declined slowly to 37% of control values after incubation for 3 h. Combined incubations with chondroitinase ABC and heparinase reduced cell surface apoE to similar levels, suggesting that it is the glycosaminoglycan-associated pool of apoE that is affected by Brefeldin A. The turnover of the fast pool is estimated at $t_{1/2}$ = 15 min. In the presence of Brefeldin A, no apoE was found in the medium, indicating that the cell surface loss was the result of internalization into the cells. The effect of Brefeldin A on the levels of apoE on the HepG2 ECM was also examined (**Fig. 3**). With Brefeldin A present, ECM-bound apoE declined slowly with a $t_{1/2}$ greater than 20 h. Therefore, apoE of the HepG2 cell surface can be divided into both dynamic and stable populations.

The ability of heparin to reduce cell surface apoE is lost under conditions that block apoE secretion (Fig. 1). One explanation for this is that heparin acts by inhibiting the binding of newly secreted apoE to the cell surface rather than by displacing apoE from HSPG or other glycosaminoglycans. To test this, we coated Remova-Wells with purified HSPG, and then examined the effects of heparin, suramin, and chondroitin sulfates on the binding of apoE

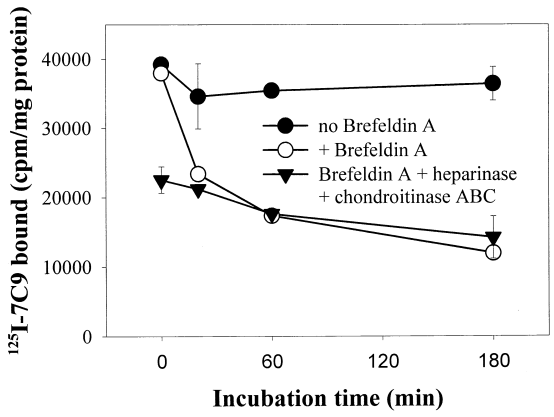

Fig. 2. Cell surface apoE decreases rapidly in the presence of Brefeldin A. HepG2 cells were incubated at 37°C with EMEM-BSA containing 2 μg/ml Brefeldin A. At the times indicated on the *x*-axis, the cells were chilled on ice and washed with cold EMEM-BSA. In some experiments, the Brefeldin A time course was preceded by sequential incubations with chondroitinase ABC (1 U/well, 30 min) and heparinase (3 U/well, 30 min). Cell surface apoE was determined as described in Experimental Procedures. All data are the mean and SE of at least three separate determinations.

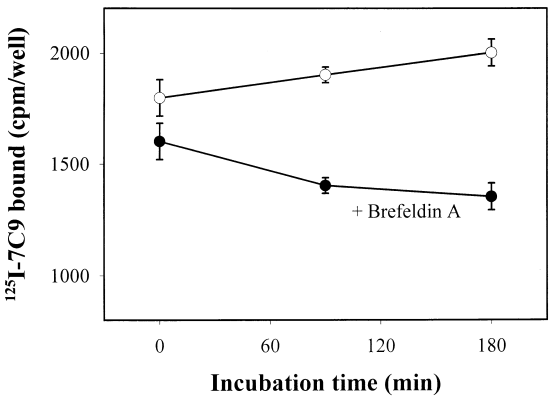

Fig. 3. The ECM represents a stable pool of cell surface apoE. HepG2 cells were incubated with Brefeldin A as described in the legend to Fig. 2. At the indicated times, the cells were chilled and washed with cold EMEM-BSA, and released from the ECM with the addition of cytochalasin (10 μg/ml) B method as described previously (16). The amount of apoE associated with the ECM was determined as described in Experimental Procedures. All data are the mean and SE of three separate determinations.

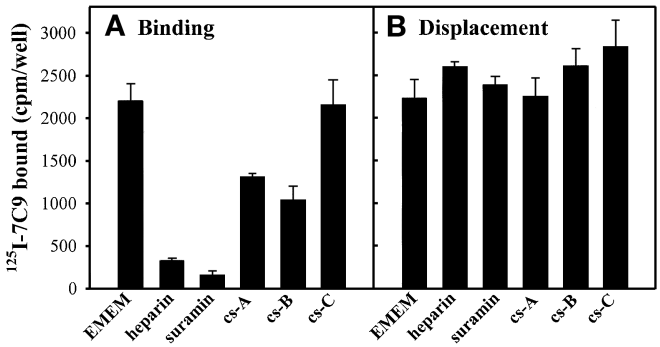

Fig. 4. Heparin, suramin, and chondroitin sulfates A and B decrease binding of apoE to HSPG, but do not effect displacement of apoE. HSPG was passively adsorbed to 96-well microtiter plates, and washed and blocked as previously described (17). To examine the effects of glycosaminoglycans on the binding of apoE to HSPG (panel A), apoVLDL (2.5 μg/100 μl) was incubated at 37°C for 2 h with the immobilized HSPG in the absence or presence of heparin (1 mg/ml), suramin (0.5 mg/ml), or chondroitin sulfates A, B, or C (1 mg/ml). The wells were washed, and ^{125}I-7C9 (100,000 cpm/well) was added and incubated for an additional 2 h. The wells were then washed with PBS and counted. To examine glycosaminoglycan-mediated displacement of apoE from HSPG (panel B), apoE was bound to the immobilized HSPG as above, and washed twice to remove unbound material. The wells were incubated at 37°C for 2 h in the absence or presence of heparin (1 mg/ml), suramin (0.5 mg/ml), or chondroitin sulfates A, B, or C (1 mg/ml). The wells were then washed with PBS and counted. All data are the mean and SE of six separate determinations.

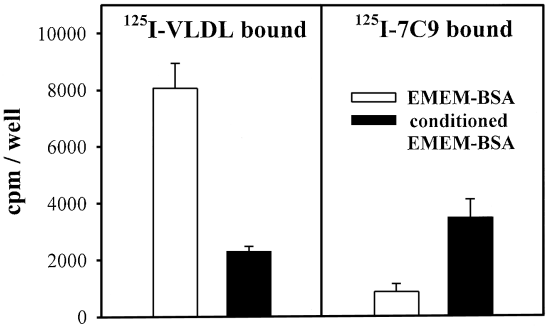

Fig. 5. ECM-bound apoE reduces binding of VLDL to the ECM. HepG2 cell-derived ECM was prepared in 12-well culture plates by the Triton X-100 method (16). ECM was incubated at 37°C for 2 h with unconditioned (open bars) or conditioned EMEM-BSA (solid bars). The ECM was washed twice with EMEM-BSA, and incubated at 37°C for 1 h with radiolabeled VLDL (100,000 cpm/ml representing about 30 ng protein; left panel) or radiolabeled 7C9 (right panel) as described in Experimental Procedures. The wells were washed, and ECM-bound counts were solubilized with 0.5 N NaOH and counted. All data represent the mean and SE of three separate determinations.

to or the displacement of apoE from the immobilized glycosaminoglycan. For this experiment, lipid-free apoE was used, and the levels associated with the ECM were determined with ^{125}I-7C9. **Figure 4** demonstrates that the binding of apoE to HSPG is efficiently inhibited by all sulfated sugars, with the exception of chondroitin sulfate C (Fig. 4A). Suramin and heparin are the most efficient in this regard, reducing binding to 15.7% and 6% of the controls, respectively. Chondroitin sulfates A and B significantly reduced the association of apoE with HSPG, indicating that these types of studies lack specificity. In contrast, when lipid-free apoE is bound to HSPG, none of these reagents promote its displacement (Fig. 4B).

ApoE bound to either the ECM or cell surface reduces lipoprotein binding

Preparation of ECM from HepG2 cells by the Triton X-100 method yields ECM with very low endogenous apoE levels (16). We have used Triton X-100 ECM to examine the effects of ECM-bound apoE on lipoprotein retention by this cell fraction. Triton X-100 ECM was incubated with either conditioned EMEM-BSA from HepG2 cells or unconditioned EMEM-BSA. Incubation with conditioned medium decreased VLDL binding to the ECM by 3-fold relative to the EMEM-BSA controls (**Fig. 5**, left panel). Conditioned medium also increased apoE on the ECM by 4-fold (Fig. 5, right panel).

We conducted similar studies with HepG2 monolayers that used heparin and suramin to decrease cell surface apoE. Prior incubations at 37°C with heparin or suramin decreased cell surface apoE by 24% and 41%, respectively (data not shown) and allowed the cells to metabolize any endogenous lipoproteins associated with the cell surface. Subsequently, the cells were washed and chilled on ice prior to the addition of ^{125}I-VLDL. Incubations with heparin increased the binding of VLDL to the cell surface by 40% (**Fig. 6**) compared with control cells. Incubations with suramin increased VLDL binding by 2.2-fold in this experiment. Collectively, these results indicate a negative correlation between cell surface or ECM-bound apoE and the binding of apoE-containing lipoproteins.

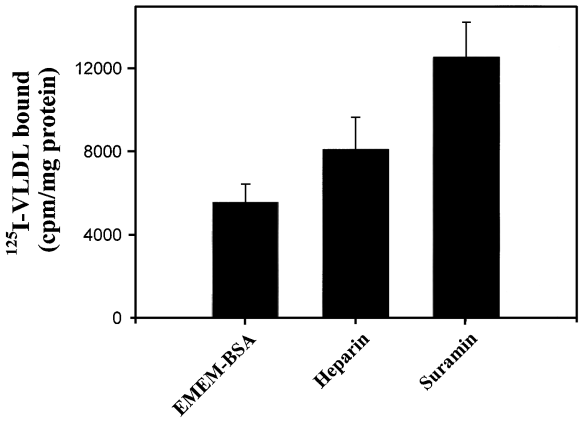

Fig. 6. Pre-incubations with heparin and suramin decrease the binding of VLDL to the HepG2 cell surface. HepG2 cells were incubated at 37°C for 1 h with EMEM-BSA (control), heparin (1 mg/ml), or suramin (0.5 mg/ml). The cells were washed three times with PBS-BSA, and incubated at 4°C for 2 h with ^{125}I-VLDL (2×10^6 cpm, 50 μg protein). The cells were then washed three times with cold PBS containing 1.7 mM $CaCl_2$, solubilized overnight in 1 ml of 0.5 N NaOH, and counted. The data are the mean and SE of three separate determinations.

Cell surface apoE can be donated to newly secreted lipoproteins

To determine whether cell surface apoE can be transferred to secreted lipoproteins, suramin was used to reduce cell surface apoE, and then secreted lipoproteins were examined for apoE content. In this study, incubation of HepG2 cells with suramin (1 h at 37°C) reduced cell surface apoE by about 50%. After this incubation, the cells were washed to remove suramin and released apoE, and fresh EMEM-BSA was added to initiate a secretion time course. We collected medium at 1 h and 3 h, and examined the amounts of apoE and apoB secreted by the cells**. Figure 7** demonstrates that the initial pre-incubation with suramin did not affect the overall secretion of apoE (top panel) or apoB (center panel) to the medium. Newly secreted apoB-containing particles were captured in microtiter wells that had been pre-adsorbed with 1D1 antibody (a monoclonal against apoB), and the relative amount of apoE associated with these particles was determined with the addition of radiolabeled 7C9. The apoE content of the lipoproteins from the suramin-treated cells was reduced to about 50% of the EMEM-BSA controls at 1 h and by about 30% after 3 h (Fig. 7, bottom panel). This reduction best corresponds with the levels of cell surface apoE after suramin treatment rather than the levels of apoE secreted to the medium (Fig. 7, top panel). To verify that the pretreatment with suramin did not enhance binding of an apoE-enriched apoB-lipoprotein to the cell surface, thereby decreasing that observed in the extracellular medium, we determined the relative amount of apoB associated with the cell surface at the 1-h and 3-h post-suramin time points. However, we found no increase in the amount of ^{125}I-1D1, an antibody against apoB, bound to the cell surface (data not shown).

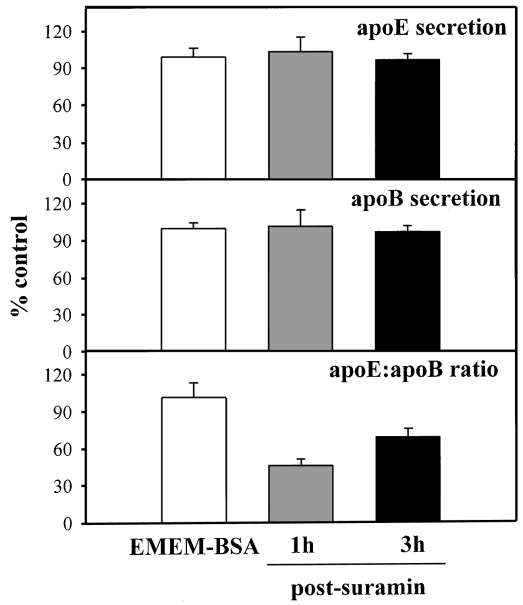

Fig. 7. Incubations with suramin decrease the apoE content of secreted apoB-containing lipoproteins. Confluent HepG2 cells in 12-well plates were incubated with EMEM-BSA (control, open bars) or 0.5 mg/ml suramin (shaded bars) for 1 h at 37°C. The cells were washed and fresh EMEM-BSA (1 ml/well) was added. The cells were incubated at 37°C for either 1 h or 3 h, and the medium samples were analyzed by radioimmunoassay for apoE (top panel) and apoB levels (center panel). To determine the apoE content of secreted apoB-containing lipoproteins, these particles were captured with 1D1 and probed with ^{125}I-labeled 7C9 (bottom panel). All data are the mean and SE of six separate determinations.

DISCUSSION

The results presented here introduce novel findings regarding cell surface apoE including *i*) a dynamic pool of cell surface apoE exists on the HepG2 cell surface and can be transferred to extracellular lipoproteins, *ii*) apoE associated with glycosaminoglycans reduces the subsequent binding of apoE-enriched lipid particles, *iii*) a stable pool of apoE associated with the ECM reduces unproductive binding of apoE-enriched particles to this surface, and *iv*) cell surface apoE can be transferred to newly secreted apoB-lipoproteins.

Studies with Brefeldin A indicate that the HepG2 cell surface maintains both dynamic and stable pools of apoE. Addition of Brefeldin A promoted a rapid decrease of cell surface apoE to about 50% of control levels after 1 h of treatment (Fig. 2), indicating that this dynamic pool must be constantly replenished from an intracellular store. The dynamic fraction of apoE appears associated with cell surface elements such as glycosaminoglycans for the following reasons: first, combined incubations with heparinase and chondroitinase ABC reduce cell surface apoE to similar levels as Brefeldin A; second, inclusion of Brefeldin A to the heparinase and chondroitinase ABC digestion resulted in no further decrease in cell surface apoE compared with the enzymatic digestions alone. In the presence of Brefeldin A, the dynamic pool is reduced by half in about 15 min. However, this rate of turnover is much faster than that reported for HSPG [$t_{1/2}$ of 2–4 h (29)], and suggests coupling with another rapidly cycling cell surface component. Preliminary studies indicate that inclusion of the receptor-associated protein (RAP) to the medium fully inhibits the rapid disappearance of cell surface apoE in the presence of Brefeldin A (J. W. Burgess and Y. L. Marcel, unpublished observations). Therefore, LDL receptor-related protein (LRP) and/or LDL receptor as shown recently in macrophages (30) may drive the rapid turnover of cell surface apoE. The more stable pool of cell surface apoE resides on the ECM. This fraction represents about 24% of apoE on the HepG2 cell surface, is lipid-poor, and has been subdivided into lipid-releasable and HSPG-associated apoE (16). The $t_{1/2}$ of greater than 20 h for this fraction corresponds well with that of other ECM proteins such as collagen and fibronectin (31, 32). Interestingly, heterogeneous pools of cell surface apoE have also been described in J774 cells transfected with human apoE3 (21). This heterogeneity includes high and low molecular weight forms representing complete and incomplete processing of the carbohydrate side chains. The low molecular weight form predominates on the cell surface and serves as a precursor

to the high molecular weight form in a conversion that requires transport through the Golgi apparatus. The low molecular weight form contributes to both dynamic ($t_{1/2}$ of 12 min) and stable pools of cell surface apoE. It was suggested that the stable pool in these cells represents a fraction of cell surface apoE that either has a longer residence time on the cell surface or undergoes repeated recycling. In this regard, a pathway that recycles apoE after lipoprotein uptake has been described in mouse hepatocytes (33) and HepG2 cells (34).

Several reports indicate that cell surface HSPG function in cooperation with apoE and the LRP in the cellular uptake of lipoproteins (2, 35, 36), although there is some dispute that the hepatic localization of apoE is essential for chylomicron remnant uptake by mouse liver (2, 36, 37). According to the secretion-recapture model, remnant lipoproteins are enriched with apoE in the Space of Disse, which promotes docking of the lipoproteins with HSPG and subsequent internalization by the LRP (2). The origin of the apoE implicated in enriching remnant particles has received a great deal of attention, and recent reports suggest that apoE, at least for LRP-dependent clearance, must derive from the hepatocyte cell surface. In this regard, bolus injections of remnants into transgenic mice expressing apoE significantly reduce the density of apoE on the hepatocyte cell surface with coincident increases in apoE associated with intracellular vesicles (15). The studies of Linton et al. (22) clearly indicate that LRP-dependent clearance of remnants absolutely requires hepatic apoE. Fazio et al. (33) also provided evidence for the heterogeneity of remnant uptake pathways and for the recycling of apoE in mouse liver. Recent observations in murine macrophages have shown that exogenously supplied apoE and endogenously synthesized apoE are confined to separate cellular compartments and may mediate distinct intracellular trafficking pathways (38). We also considered the possibility that the apoE content of cell surface glycosaminoglycans might affect the ability of cells to bind apoE-containing lipoproteins. We found that incubations of HepG2 cells with heparin or suramin, although reducing cell surface apoE by 25% and 50%, respectively, increased the binding of VLDL by 1.5- and 2.3-fold, respectively (Fig. 6). Our data also indicate that increased apoE association with the ECM reduces the binding of VLDL (Fig. 5). Collectively, our data indicate that glycosaminoglycan-associated apoE can occlude binding sites for apoE-containing lipoproteins on the cell surface. Minimizing associations of apoE-containing lipoproteins with HSPG of the ECM would minimize binding to sites not directly linked to internalization, and allow unimpeded movement of the lipoproteins through the Space of Disse and to cell surface HSPG and LRP. Other glycosaminoglycan-binding proteins (e.g., hepatic lipase) could similarly reduce the binding of lipoproteins to cells or ECM, providing that their binding sites on glycosaminoglycans overlap with apoE.

ApoE is associated with the newly secreted VLDL in model hepatocytes (39), and the hepatic synthesis of apoE contributes to the regulation of VLDL triglyceride secretion by the liver (40, 41). However, it is still unclear where in the secretion pathway apoE becomes associated with VLDL. Our studies suggest that significant amounts of apoE can be transferred directly from the HepG2 cell surface to secreted apoB-containing lipoproteins. In these studies, we used suramin to reduce the apoE content of the cell surface, and then examined the apoE content of endogenously secreted apoB particles. Suramin decreased cell surface apoE and particle-associated apoE by 42% and 44%, respectively, but had no effect on the release of apoE or apoB to the extracellular medium (Fig. 7). This suggests that cell surface apoE can be used to enrich the apoE content of newly secreted lipoprotein particles. Arguments based on pool size indicate that the suramin-affected pool of cell surface apoE and that which is transferred to secreted lipoproteins originates, at least in part, from CSPG. In this regard, we reported previously that the majority of cell surface apoE in HepG2 cells is associated with CSPG (17). Assuming a weak interaction between apoE and the poorly sulfated CSPG, we suggest that this fraction of cell surface apoE could be transferred to newly secreted lipoproteins or even to incoming remnants. The concept that cell surface apoE can be transferred to extracellular lipoproteins is supported by the findings of Schmitt and Grand-Perret (20). These authors demonstrated decreases in the levels of cell surface apoE following incubations of HepG2 cells with LDL and HDL at 4°C. Therefore, the newly synthesized VLDL acquires exchangeable apolipoproteins such as apoE not only intracellularly in the Golgi as shown by Fazio and Yao (12), but also from the cell surface pool as it emerges out of the hepatocytes.

The studies with heparin, suramin, and the chondroitin sulfates indicate that apoE cannot be displaced from glycosaminoglycans when bound (Fig. 4), and that suramin may reduce cell surface apoE by inhibiting the binding of newly secreted apoE to glycosaminoglycans. The actions of these small sulfated glycosaminoglycans in cells would be analogous to that of Brefeldin A, in that replenishment of the dynamic cell surface pool would be inhibited. That incubations with heparin or suramin at 37°C decrease cell surface apoE indicates that the presentation of apoE at the cell surface involves either binding of medium apoE to surface glycosaminoglycans or transfer of newly synthesized apoE from a co-secreted element to cell surface glycosaminoglycans.

In **Fig. 8**, we present a model for the involvement of cell surface apoE in the metabolism of chylomicron remnants. Newly synthesized apoE or apoE derived from a recycling pool (step 1) is transported to cell surface CSPG (step 2). The apoE-CSPG complex is a low affinity association and allows the transfer of apoE to either cell surface HSPG (step 3A) or to available remnant particles (step 3B). Lipid-poor apoE of the ECM functions to minimize unproductive binding of the apoE-enriched remnants to the ECM (step 4), thereby facilitating their association with plasma membrane HSPG and LRP (step 5) and subsequent internalization (step 6).

In conclusion, apoE of the HepG2 cell surface comprises both dynamic and stable fractions. The replenish-

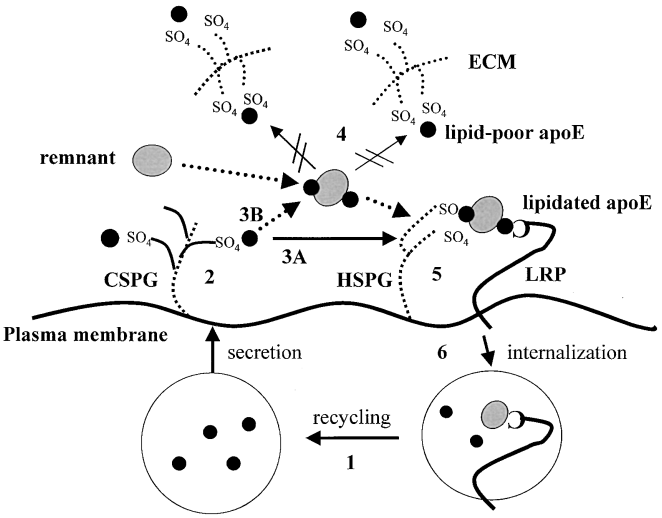

Fig. 8. Summary of remnant uptake by cell surface apoE and the HSPG-LRP pathway. The apoE from newly synthesized stores or from a recycling pool (step 1) is transported to cell surface CSPG (step 2). In the absence of remnant particles, apoE is transferred to HSPG and internalized by the HSPG-LRP pathway with a $t_{1/2}$ of about 15 min (step 3A). When remnants are present, apoE is transferred to the lipid particles (step 3B). Lipid-poor apoE of the ECM minimizes unproductive binding of the apoE-enriched remnants to the ECM (step 4), facilitating their association with plasma membrane HSPG and LRP (step 5) and subsequent internalization (step 6).

ment of the rapidly turning-over pool of apoE requires a functional Golgi apparatus, and is distinct from the slow pool on the ECM. We propose that one of the functions of the ECM pool is to prevent unproductive binding of lipoproteins. The rapidly turning-over pool of apoE at the cell surface contributes to both uptake and secretion of lipoproteins by the hepatocyte.

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