Interaction of a recombinant form of apolipoprotein[a] with human fibroblasts and with the human hepatoma cell line HepG2

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Abstract We have studied the binding, uptake, and degradation of a recombinant form of apolipoprotein[a] (r-apo[a]) using a cultured cell model. In HepG2 cells and in human fibroblasts, r-apo[a] complexed with low density lipoprotein (LDL) is bound and internalized via high affinity (K_d = 10 nM) receptors; in both cell types, low affinity (K_d = 200–300 nM) sites also mediate free apo[a] uptake. Using competition studies, we found that the high affinity binding component corresponds to the LDL receptor. Involvement of the LDL receptor in r-apo[a] uptake by fibroblasts was confirmed using fibroblasts derived from an individual homozygous for familial hypercholesterolemia; in contrast to normal fibroblasts, these cells lacked the high affinity r-apo[a] binding component. Cell association of ^{125}I-labeled r-apo[a] was increased and decreased concomitantly with the up- and down-regulation of the LDL receptor in response to a number of compounds. The addition of α_2-macroglobulin as well as treatment with heparinase, chondroitinase ABC, and sodium chlorate did not decrease total specific binding of r-apo[a], suggesting that neither the low density lipoprotein receptor-related protein nor cell surface proteoglycans are involved in r-apo[a] clearance. The low affinity binding component present in both fibroblasts and HepG2 cells likely corresponds to the plasminogen receptor, as binding of r-apo[a] to these sites was specifically decreased by the addition of plasminogen or the lysine analogue ε-aminocaproic acid, but not by the addition of tissue-type plasminogen activator. Heparin abolished uptake of r-apo[a] by the LDL receptor component only; this indicates that apo[a] must be associated with LDL to be cleared by this receptor. In contrast, free apo[a] can be effectively cleared by the plasminogen receptor which may represent a significant route of clearance for free apo[a] in vivo.–**Tam, S-P., X. Zhang, and M. L. Koschinsky.** Interaction of a recombinant form of apolipoprotein[a] with human fibroblasts and with the human hepatoma cell line HepG2. *J. Lipid Res.* 1996. **37:** 518–533.

Supplementary key words lipoprotein[a] • low density lipoprotein receptor • low density lipoprotein receptor-related protein • plasminogen receptor

Lipoprotein[a] (Lp[a]) is a highly studied plasma lipoprotein owing to the relationship that has been demonstrated between elevated levels of Lp[a] and the development of coronary heart disease and stroke in human populations (see ref. 1 for a recent review). Lp[a] is distinguishable from LDL by the presence of apolipoprotein[a] (apo[a]), which is covalently linked to the apolipoprotein B-100 (apoB-100) moiety of LDL by a single disulfide bridge (2). Recent reports strongly suggest that Lp[a] particle assembly occurs extracellularly, in plasma (2, 3).

Apo[a] consists of multiple repeated copies of a sequence closely resembling plasminogen kringle IV, followed by sequences that are highly similar to the kringle V and protease domains of plasminogen (4). The similarity between apo[a] and plasminogen suggests that interference with the normal fibrinolytic functions of plasminogen may be a mechanism for the atherogenic effects of Lp[a]. In this context, it has been shown that Lp[a] binds to plasminogen receptors present on endothelial cells and on monocytes (5, 6) as well as to fibrin (7, 8).

By virtue of its similarity with LDL, it has been proposed that Lp[a] can effectively compete with LDL for binding and/or uptake by the LDL receptor. There has been a great deal of controversy concerning the ability

Abbreviations: Lp[a], lipoprotein[a]; LDL, low density lipoprotein; apo[a], apolipoprotein[a]; tPA, tissue-type plasminogen activator; α_2M, α_2-macroglobulin; LRP, low density lipoprotein receptor-related protein; FH, familial hypercholesterolemia; NaN_3, sodium azide; ε-ACA, ε-aminocaproic acid; HDL, high density lipoprotein; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene-bis(oxyethylenenitrilo) tetraacetic acid; HEPES, [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]; ACAT, acyl-coenzyme A:cholesterol acyltransferase.

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of Lp[a] to interact with the LDL receptor, and the possible role of this receptor in Lp[a] clearance from the circulation. Several studies in tissue culture and in vivo suggest that the LDL receptor plays a role in the binding and catabolism of Lp[a] (9–11), while other studies suggest that this is not a significant route for Lp[a] clearance due to reduced affinity of Lp[a] for this receptor (12, 13). Interestingly, Hofmann et al. (14) demonstrated that transgenic mice which overproduce the LDL receptor can rapidly clear human Lp[a] from the circulation. Most recently, it has been shown that nonspecific degradation of Lp[a] in vitro varies greatly, depending on the cell type used (15); this has been suggested to result from a variable contribution of the LDL receptor to Lp[a] clearance in different cell types.

In addition to the LDL receptor, the role of other potential Lp[a] receptors in the binding and uptake of this lipoprotein has been studied. The LDL receptor-related protein/α_2-macroglobulin receptor (LRP/α_2MR) is a recently described multifunctional receptor which is present in a wide variety of cells and tissues. This receptor binds to apolipoprotein E (apoE)-enriched lipoproteins (16, 17) and may clear chylomicron remnants from the circulation by an LDL receptor-independent pathway (18). This receptor also binds several non-lipoprotein ligands such as activated α_2-macroglobulin (α_2M) (19) as well as type-1 plasminogen activator inhibitor/urokinase plasminogen activator (PAI-1/uPA) complexes (20). Owing to its structural similarity with the LDL receptor, a role for the LRP in Lp[a] clearance has been postulated. Recent data have been presented suggesting that the LRP may be involved in the internalization of high molecular weight Lp[a] species (21).

In addition to the LRP, plasminogen receptors, which play a key role in hemostasis, are also found in a wide variety of cell types (22). In one study utilizing HepG2 cells, incubation of apo[a] (obtained by reduction of Lp[a]) with the cells in the presence or absence of plasminogen did not alter the amount of LDL associated with the cells (23), suggesting that the plasminogen receptor does not significantly contribute to binding of apo[a] by HepG2 cells.

In the present study, we directly examined the ability of a recombinant form of apo[a] to interact with receptors present on cultured human hepatoma cells and human foreskin fibroblasts. Our results suggest that apo[a] is not a ligand for the LDL receptor present on the HepG2 cells, but when complexed with LDL, can be effectively bound and internalized by this route. Uncomplexed recombinant apo[a] was shown to bind to lower affinity sites present on both HepG2 cells and human fibroblasts, which likely represent receptors for plasminogen.

MATERIALS AND METHODS

Purification of proteins

Lipoproteins were isolated from freshly drawn citrated human blood. Disodium EDTA, pH 7.3, and NaN_3 were added to plasma to final concentrations of 0.01% (w/v) and 0.02% (w/v), respectively. Density gradient ultracentrifugation was used to isolate LDL (d 1.019–1.063 g/ml), HDL (d 1.063–1.21 g/ml), and heavy HDL (d 1.16–1.21) by a modification (24) of the method of Havel, Eder, and Bragdon (25) in a Beckman L8-70 ultracentrifuge using an SW27 rotor. Lipoprotein-depleted serum (LPDS) was prepared by adjustment of the serum density to 1.21 g/ml with NaBr followed by the removal of lipoproteins by ultracentrifugation.

A recombinant form of apolipoprotein[a] (r-apo[a]) containing 17 kringle IV domains, as well as the kringle V and protease domains (26) was purified from stably transfected human embryonic kidney cells by gel exclusion chromatography using Sepharose CL-4B (Pharmacia). As we have demonstrated previously (26), although the predicted molecular mass of r-apo[a] is 250 kDa, the apparent molecular weight on SDS-PAGE is ~525,000.

An Lp[a]-like complex was formed by incubating LPDS with LDL (10 μg/ml) and ^{125}I-labeled r-apo[a] (10 μg/ml) at 37°C for 2 h, followed by isolation between d 1.03 to 1.05 g/ml.

Plasminogen (i.e., native Glu^1-plasminogen) was purified from fresh-frozen human plasma by adsorption to lysine-Sepharose CL-4B, followed by elution with 20 mM ε-aminocaproic acid (ε-ACA). The dialyzed sample was precipitated with 70% ammonium sulfate and the precipitate was dissolved in a minimal volume of 50% glycerol. α_2-Macroglobulin (α_2M) was purchased from CalBiochem.

Cell culture

Human hepatoma (HepG2) cells (27) were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) as described previously (28). Human fibroblasts were derived from a normal individual and an individual homozygous for familial hypercholesterolemia (FH) (kindly provided by Dr. Ross Milne, Ottawa Heart Institute); both cell types were cultured in the same medium as the HepG2 cells. All cell types were grown to sub-confluency in 24-well tissue culture plates in serum-supplemented media; before each experiment cells were preconditioned by an 18-h incubation in MEM containing 10% LPDS (d > 1.21 g/ml) or 10% FBS plus various supplements as indicated. Cell viability was routinely monitored by trypan blue exclusion and lactate dehydrogenase leakage

as described previously (29). In all experiments, the number of nonviable cells never exceeded 5% of the total cell number.

Radiolabeling of lipoproteins and proteins

LDL, recombinant apo[a], ovalbumin, and α_2M were iodinated according to the iodine monochloride procedure of McFarlane (30). 0.2 mCi of $Na^{125}I$ was used to iodinate 1 mg of protein in the presence of 40 nmol of iodine monochloride and 0.5 M glycine–NaOH buffer, pH 10. Free iodine was removed by gel filtration on Sephadex G-50 (Sigma), followed by overnight dialysis in saline containing 0.01% EDTA and 0.02% NaN_3, pH 7.4. All iodinated lipoproteins and proteins were extensively dialyzed against sterile saline prior to use. The specific radioactivities for LDL, r-apo[a], ovalbumin, and α_2M were 120–150 cpm/ng protein, 40–60 cpm/ng protein, 100–120 cpm/ng protein, and 500–600 cpm/ng protein, respectively.

Cell association of LDL and α_2-macroglobulin

At 18 h prior to the experiment, the medium of the HepG2 cell cultures grown in multiwell dishes was replaced with medium containing either 10% LPDS or 10% FBS. In some experiments, the cells were preincubated overnight with MEM plus 10% FBS containing one of the following supplements: 20 μM compactin (Sigma M 2537), 2 μM 25-hydroxycholesterol (Sigma H 1015), the Sandoz compound 58-035 (5 μg/ml), or heavy HDL (100 μg apolipoprotein A-I/ml). Prior to the experiment, the cells were washed three times with phosphate-buffered saline (PBS) and then incubated in MEM containing 0.2% bovine serum albumin (BSA) for 30 min at 37°C. The experiment was initiated by replacing the medium with 0.3 ml MEM containing 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, 0.2% BSA and ^{125}I-labeled LDL (10 μg/ml) in the presence or in the absence of either unlabeled LDL (300 μg/ml) or 1 mM EGTA. After a 3-h incubation at 37°C, the medium from each cell dish was removed. The cells were cooled on crushed ice and washed 3 times with MEM containing 0.2% BSA and twice with PBS. The cells were then dissolved in 1 N NaOH and cell-associated ^{125}I-labeled LDL was measured. A portion of the cell lysate was used for protein determination by the method of Lowry et al. (31). To block the LDL receptor, cells were preincubated for 30 min at 37°C with the monoclonal anti-LDL receptor antibody IgG-C7 (ATCC #CRL 1691) at a concentration of 100 μg/ml (32).

Cell association of ^{125}I-labeled α_2M in HepG2 cells or human fibroblasts was also determined. Prior to the experiments, the cells were washed 3 times with PBS and then incubated in MEM containing 0.2% BSA for 30 min at 37°C. ^{125}I-labeled α_2M (10 μg/ml) in 250 μl of MEM containing 0.2% BSA, 10 mM HEPES, pH 7.4, was incubated with the cells for 3 h at 37°C. After incubation, the cells were washed 3 times with PBS containing 0.2% BSA and once with PBS alone. Subsequently the cells were dissolved in 1 N NaOH; this solution was counted for radioactivity and measured for protein content (31). Nonspecific binding was determined in the presence of a 50-fold molar excess of unlabeled ligand. In some cases, HepG2 cells and human fibroblasts were also preincubated for 30 min at 37°C with the monoclonal antibody IgG-5D7 (ATCC #CRL 1938) that recognizes the external domain of the LRP (33) in order to determine whether or not this monoclonal antibody could inhibit LRP/α_2MR activity.

Binding, cell association, and degradation of r-apo[a]

Binding and uptake experiments were performed in 24-well tissue culture plates using 90–95% confluent monolayers of cells. Prior to each experiment, HepG2 cells or human fibroblasts were incubated overnight in MEM containing various supplements as indicated. For binding experiments, cells were prechilled on crushed ice for 30 min. The medium was removed and the cells were incubated for 3 h at 4°C with various concentrations of radiolabeled r-apo[a] in MEM containing 10 mM HEPES (pH 7.4) and 1% BSA. Nonspecific binding was determined by the addition of a 50-fold molar excess of unlabeled r-apo[a] at each concentration as specified in the figure legends. Binding reactions were terminated by removing the medium; cells were subsequently washed twice with 2 ml of ice-cold PBS containing 0.2% BSA, and twice with PBS alone. The cells were then dissolved in 1 N NaOH and the lysates were transferred to scintillation vials for determination of radioactivity. Specific binding was calculated by subtracting the nonspecific binding from total binding. In some experiments, the binding studies were carried out in the presence of increasing amounts of unlabeled r-apo[a], α_2M, plasminogen, tissue-type plasminogen activator (tPA), LDL, HDL, and a combination of LDL and plasminogen as described in the figure legends.

In some studies, cell association of ^{125}I-labeled r-apo[a] in HepG2 cells and human fibroblasts was also determined. Immediately prior to these experiments, the cells were washed 3 times with PBS and then incubated for 30 min at 37°C in MEM containing 0.2% BSA. ^{125}I-labeled r-apo[a] (120 nM) in 250 μl of MEM containing 10 mM HEPES (pH 7.4) and 0.2% BSA were incubated with the cells for 3 h at 37°C under various culture conditions as indicated. After incubation, the medium was removed and the cells were washed 3 times with PBS containing 0.2% BSA followed by one wash with PBS alone. The washed cells were dissolved in 1 N NaOH. The radioactivity in the cell lysates represents the total

amount of ^{125}I-labeled r-apo[a] that became cell-associated. A portion of the cell lysate was used for protein determination (31). Nonspecific binding was determined in the presence of a 50-fold molar excess of unlabeled r-apo[a].

Cellular uptake and degradation of r-apo[a] was determined by incubating HepG2 cells or human fibroblasts with varying concentrations of ^{125}I-labeled r-apo[a] for 6 h at 37°C, in the absence or presence of a 50-fold molar excess of unlabeled r-apo[a]. At this time, the medium was removed and the cells were washed with PBS as described above. To determine the amount of ^{125}I-labeled r-apo[a] bound to the cell surface, monolayers were treated with 1 ml of 0.05% (w/v) trypsin and incubated for 4 min at 37°C as described by Stein et al. (34). The cells were then centrifuged at 2000 rpm for 10 min at 4°C. The radioactivity released by trypsin is a measure of bound r-apo[a], while that in the cell pellet represents the internalized r-apo[a]. These fractions, as well as the culture medium, were subsequently incubated with 10% trichloroacetic acid (TCA) to assess soluble and precipitable radioactivity. Degradation was determined by measurement of both total TCA-soluble radioactivity released to the medium and that inside the cells. The TCA-soluble radioactivity was further analyzed for water-soluble (presumably small peptides and amino acids) and chloroform-soluble (iodine) radioactivity as described by Bierman, Stein, and Stein (35). Cellular uptake and degradation of ^{125}I-labeled ovalbumin by human fibroblasts was determined in a manner similar to that described for r-apo[a]. In some experiments, 10 mM EDTA or EGTA was added to the medium together with labeled r-apo[a] to assess Ca^{2+}-dependent binding. To inactivate lysosomal proteases, cells were preincubated for 30 min at 37°C with 100 μM chloroquine; this concentration of chloroquine was maintained after the addition of ^{125}I-labeled r-apo[a]. Specific anti-apoB polyclonal antibodies (100 μg/ml) (36) were used to prevent any apoB-associated particles or complexes from interacting with the LDL receptors. In some experiments, heparin (10 mg/ml) was used to remove any residual LDL associated with LDL receptors prior to the interaction of r-apo[a] with HepG2 cells (37). Cellular uptake and degradation of r-apo[a] was also studied in the presence of 10 mM ε-aminocaproic acid (ε-ACA). Binding of ^{125}I-labeled r-apo[a] was also performed in the presence of ε-ACA at concentrations ranging from 2 to 50 mM.

Treatment of cells with heparinase, chondroitinase ABC, and sodium chlorate

HepG2 cells and human fibroblasts were cultured as described above. Conditions for the use of heparinase III (Sigma H 8891) and chondroitinase ABC (Sigma C 2905) were based on the procedures described by Ji et al. (38) and Oike et al. (39), respectively. Briefly, the cells were washed 4 times at 37°C in PBS and then incubated for 2 h at 37°C with either heparinase (2 units/ml) or chondroitinase (0.25 units/ml) in MEM containing 0.2% BSA, 25 mM HEPES (pH 7.4). The cells were washed an additional 4 times with PBS at 37°C and ^{125}I-labeled r-apo[a] cell association was assessed as described above. In some experiments, the cells were preincubated for 48 h with 30 mM sodium chlorate prior to cell association experiments. To avoid the possibility that the cell surface proteoglycans might regenerate once heparinase, chondroitinase, or sodium chlorate was removed, we also determined ^{125}I-labeled r-apo[a] binding to the cells at 4°C.

Other methods

The level of human apoB secreted by HepG2 cells was determined by radioimmunoassay as described previously (28). Cellular protein content was determined by the method of Lowry et al. (31).

RESULTS

Binding of recombinant apo[a] to cultured human hepatoma cells

The binding of ^{125}I-labeled r-apo[a] (120 nM) to HepG2 cells at 4°C appeared to be saturable as a function of time, reaching a plateau within 3 h (**Fig. 1**). The total binding of ^{125}I-labeled r-apo[a] to the HepG2 cells was inhibited by approximately 75% in the presence of a 50-fold molar excess of unlabeled r-apo[a].

The HepG2 cells were then incubated with increasing concentrations of ^{125}I-labeled r-apo[a] for 3 h at 4°C. Specific binding of r-apo[a] to cultured HepG2 cells preincubated with LPDS is shown in **Fig. 2.** The shape of the curve suggested the presence of at least two binding components of different affinities. Analysis of the specific binding by the method of Scatchard yielded a non-linear plot (Fig. 2, inset). The plot was resolved into two components as described by Rosenthal (40); one of these had a K_d of 10 nM, and the other a K_d of 305 nM. The maximum binding capacities (B_{max}) for the high and low affinity binding sites were calculated to be 0.11 and 0.33 pmol r-apo[a]/mg cellular protein, respectively.

The role of Ca^{2+} in ^{125}I-labeled r-apo[a] binding could not be examined under optimal conditions because of the presence of Ca^{2+} (1.8 mM) in the culture medium. However, specific binding of r-apo[a] to the high affinity binding site was completely abolished in the presence of 10 mM EDTA or 10 mM EGTA (**Fig. 3**). In contrast, binding of r-apo[a] to the low affinity binding site ap-

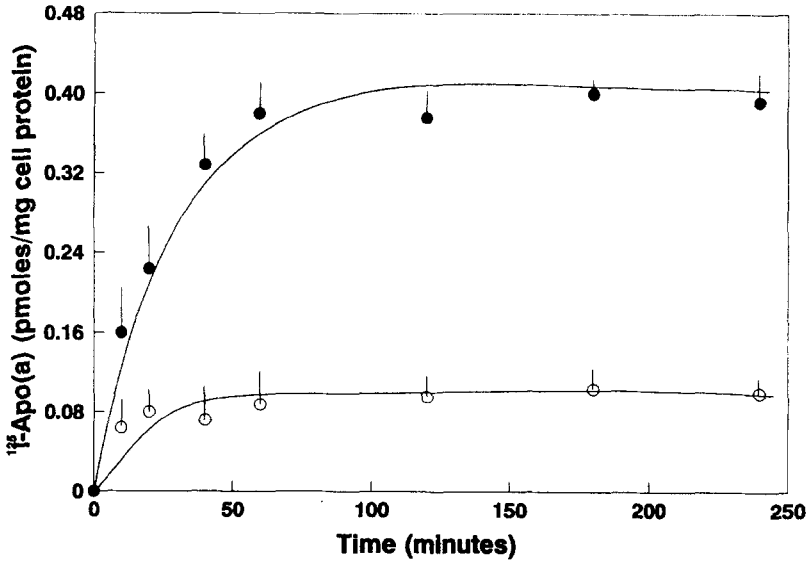

Fig. 1. Time course of ^{125}I-labeled r-apo[a] binding to the human hepatoma cell line HepG2. Subconfluent cell monolayers were incubated overnight in MEM containing 10% LPDS. For binding assays, cells (~5×10^6) were prechilled on crushed ice for 30 min. The medium was removed and the cells were incubated with a constant concentration of ^{125}I-labeled r-apo[a] (120 nM) in the absence (solid circles) or presence (open circles) of a 50-fold molar excess of unlabeled r-apo[a]. After incubation at 4°C for the indicated time, the radioactivity associated with the cells was determined. The results represent the mean ± SEM of three independent experiments.

peared to be Ca^{2+}-independent as it was unaffected by EDTA or EGTA (Fig. 3).

Specificity of binding

Competitive binding experiments were conducted to define the specificity of the two classes of r-apo[a] binding sites on HepG2 cells. Binding of ^{125}I-labeled r-apo[a] (400 nM) was inhibited by unlabeled native r-apo[a], but not by HDL or activated α_2M (**Fig. 4**). Twenty-five percent of total specific binding of ^{125}I-labeled r-apo[a] was inhibited by a 10- to 20-fold molar excess of LDL, while a 50- or 100-fold excess of LDL could only block this specific interaction by a further 15%. By contrast, a 50- or 100-fold molar excess of plasminogen was capable of inhibiting the specific binding by 40% and 80%, respectively (Fig. 4A). Furthermore, there appeared to be an additive effect when a combination of LDL and plasminogen was used as the competitor. Similar results were obtained for these competitive binding analyses using 40 nM ^{125}I-labeled r-apo[a] (Fig. 4B). The specificity of plasminogen with respect to inhibition of r-apo[a] binding was studied using recombinant tissue-type plasminogen activator (r-tPA; Genentech Inc.) which contains two kringle motifs. No competition was observed by the inclusion of up to a 100-fold molar excess of tPA using HepG2 cells (Figs. 4A and B). Taken together, the data suggest that LDL and plasminogen may be competing with r-apo[a] for binding to the high and low affinity

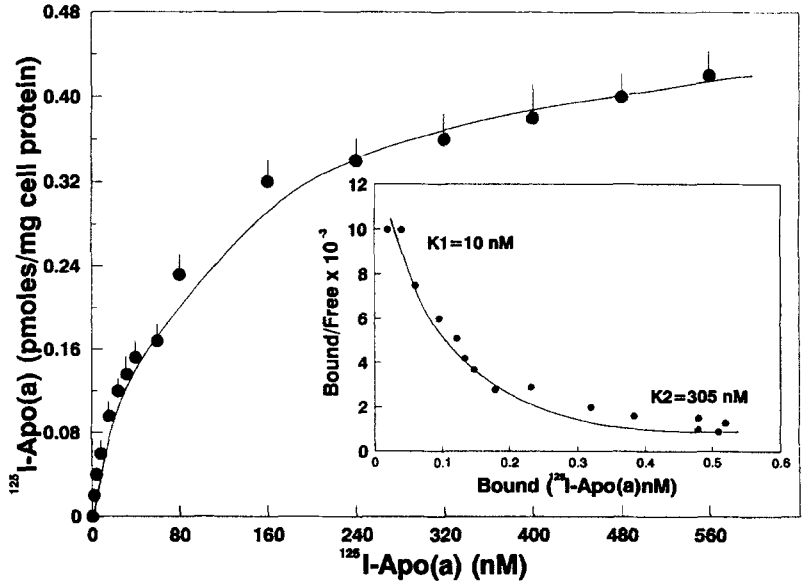

Fig. 2. Binding of ^{125}I-labeled r-apo[a] to HepG2 cells. Experiments were performed using 90–95% confluent monolayers in 24-well tissue culture plates. Before each experiment, HepG2 cells were preincubated overnight in MEM containing 10% LPDS. For binding assays, cells (~5×10^6) were prechilled on crushed ice for 30 min. The medium was removed and the cells were incubated with increasing concentrations of ^{125}I-labeled r-apo[a] (specific activity = 40–60 cpm/ng protein) in the absence or presence of a 50-fold molar excess of unlabeled r-apo[a] at each concentration indicated in the figure. Binding at 4°C was measured for 3 h as described in Materials and Methods. The curve represents specific binding (total minus nonspecific); nonspecific binding amounted to less than 15% of total binding. The inset shows the Scatchard plot of the binding curve. Dissociation constants were determined by the graphical procedure of Rosenthal (40). Results are the mean ± SEM from three independent experiments carried out in duplicate. For the purpose of simplification, only mean values are presented in the Scatchard plot (see inset).

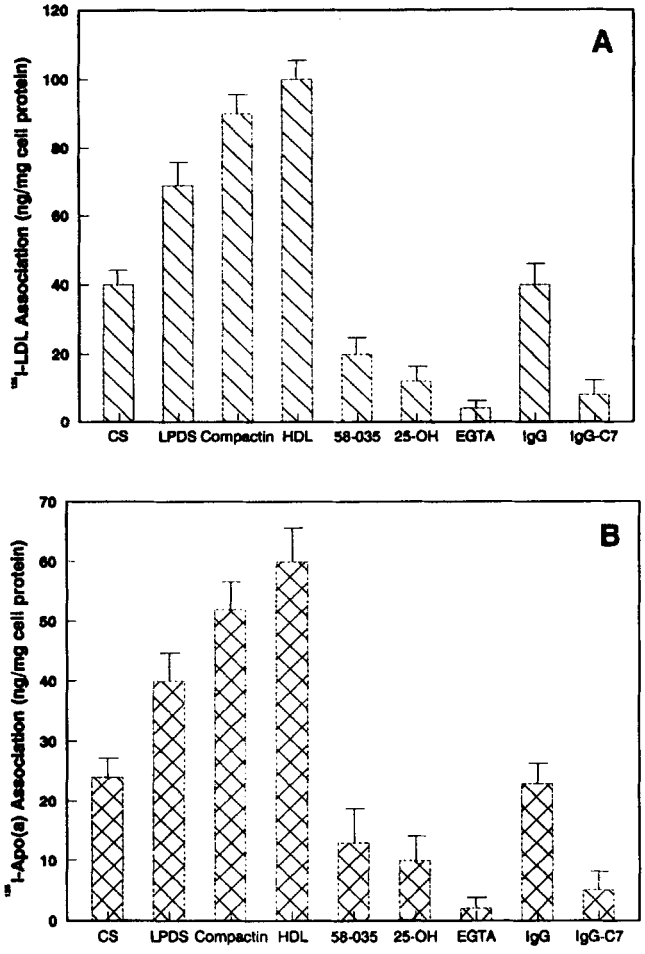

Fig. 3. Effect of up- and down-regulation of the LDL receptor as well as the anti-LDL receptor monoclonal antibody IgG-C7 on the cell association of ^{125}I-labeled LDL and ^{125}I-labeled r-apo[a] to HepG2 cells. Cell association assays (37°C for 3 h) and preincubation conditions are described in Materials and Methods. Specific cell association was calculated by subtracting total cell association from nonspecific association. Nonspecific cell association was determined by performing the assay in the presence of a 50-fold molar excess of unlabeled ligands. Panel A: specific cell association of ^{125}I-labeled LDL (10 μg/ml) to HepG2 cells. Prior to the experiments, cells were preincubated with the following supplements: 10% complete bovine serum (CS); 10% LPDS; compactin; HDL; Sandoz compound 58-035 (an ACAT inhibitor); 25-hydroxycholesterol (25-OH); 10 mM EGTA; an antibody against human serum albumin (IgG) and anti-LDL receptor monoclonal antibody (IgG-C7). Panel B: specific cell association of ^{125}I-labeled r-apo[a] to HepG2 cells. The experiments were carried out using 20 nM of ^{125}I-labeled r-apo[a] in order to avoid the interference of the low affinity component. Cells were preincubated as described in panel A. Results are the means ± SEM of three independent experiments.

sites respectively.

Effects of up- and down-regulation of the LDL receptor as well as an anti-LDL receptor antibody on cell association of ^{125}I-labeled LDL and ^{125}I-labeled r-apo[a]

It has been shown previously that LPDS (41–43), compactin (43, 44), and heavy HDL (45) can up-regulate LDL receptor activity while the Sandoz compound 58-035 (an ACAT inhibitor) (42, 46) and 25-hydroxycholesterol (40, 41) can down-regulate its activity. Fig. 3A and B demonstrate that cell association of both ^{125}I-labeled LDL (10 μg/ml) and ^{125}I-labeled r-apo[a] (20 nM) increase and decrease concomitantly with the up- and down-regulation of the LDL receptor activity. Cell association of these two radioactive ligands with the high affinity binding sites could be blocked by the addition of either 10 mM EGTA or the anti-LDL receptor monoclonal antibody IgG-C7 but not with control IgG (antibody against human serum albumin).

Effects of anti-LDL receptor antibody and ε-aminocaproic acid on the binding of ^{125}I-labeled r-apo[a] to the low affinity binding sites

HepG2 cells were pretreated with the anti-LDL receptor monoclonal antibody IgG-C7 (100 μg/ml) for 30 min at 37°C; cells were washed and chilled on ice prior to the experiments. The binding assays were carried out at 4°C for 3 h in the presence of 10 mM EDTA. **Figure 5A** demonstrates that control IgG and IgG-C7 did not affect the specific binding of ^{125}I-labeled r-apo[a] to the low affinity binding site. The effect of various concentrations of ε-ACA (0–50 mM) on ^{125}I-labeled r-apo[a] binding was also examined. It was found that ε-ACA concentrations between 10 and 50 mM abolished 80% of the specific binding of r-apo[a] to the low affinity compo-

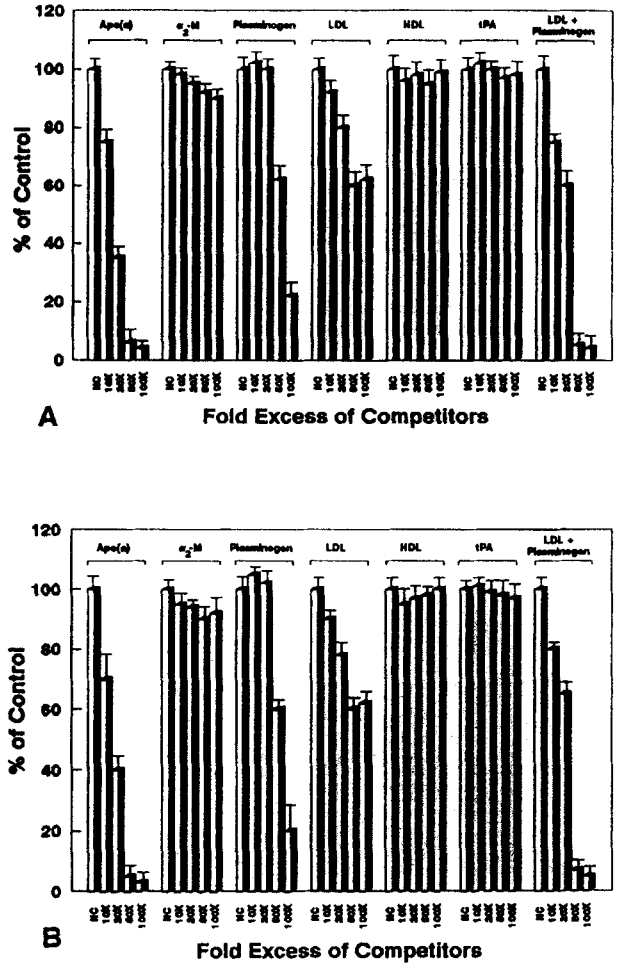

Fig. 4. Competitive binding studies. Newly confluent HepG2 cells were grown overnight in MEM containing 10% LPDS. Binding of ^{125}I-labeled r-apo[a] (400 nM, panel A or 40 nM, panel B) to the cells was measured in the presence of 10-, 20-, 50-, and 100-fold molar excesses of unlabeled r-apo[a], α_2-macroglobulin (α_2-M), plasminogen, LDL, HDL, tissue-type plasminogen activator (tPA), or a combination of equivalent molar concentrations of LDL plus plasminogen at 4°C as described in Materials and Methods. Binding in the absence of competitor (NC) was arbitrarily set at 100%. The results are the mean ± SEM of three independent experiments.

nent (Fig. 5B). Under these conditions, > 95% of the cells were viable as determined by trypan blue exclusion and lactate dehydrogenase leakage assays.

Effect of heparin and anti-apoB-100 antibodies on the specific binding of ^{125}I-labeled r-apo[a] to HepG2 cells

HepG2 cells were pretreated with heparin (10 mg/ml) in order to remove any residual LDL associated with the LDL receptors. The cells were then washed 4 times with cold PBS to remove the heparin prior to analysis of the binding of ^{125}I-labeled r-apo[a] at 4°C. **Figure 6** shows that heparin pretreatment of the cells prior to r-apo[a] addition results in a typical saturation curve for r-apo[a] binding; Scatchard analysis of this binding data resulted in a linear plot corresponding to the low affinity component only (see inset, Fig. 6).

The level of human apoB secreted by HepG2 cells is 280 ± 26 ng/h per mg cellular protein as determined by

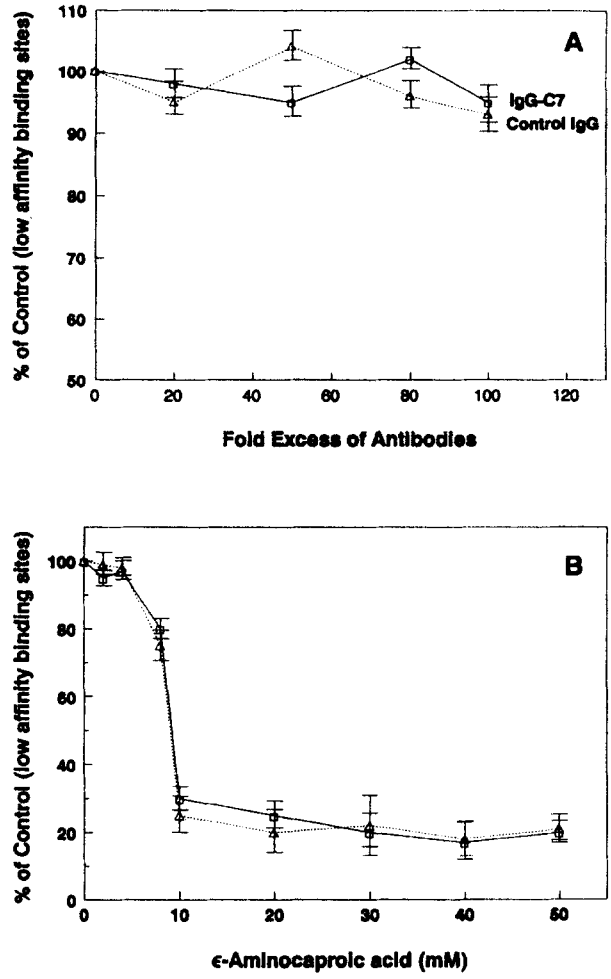

Fig. 5. Effects of anti-LDL receptor monoclonal antibody IgG-C7 and ε-ACA on the binding of ^{125}I-labeled r-apo[a] to the low affinity binding site of human hepatoma cells and fibroblasts. Binding assays were carried out at 4°C for 3 h as described in Materials and Methods. Specific binding was determined by subtracting nonspecific binding (i.e., that carried out in the presence of 50-fold molar excess of unlabeled r-apo[a]) from total binding. The binding assays for the low affinity component were carried out in the presence of ^{125}I-labeled r-apo[a] (400 nM) and 10 mM EDTA as the high affinity binding site is abolished by the removal of Ca^{2+}. Panel A: monolayers of HepG2 cells were incubated with ^{125}I-labeled r-apo[a] in the presence of varying amounts of control IgG (antibody against human albumin) (△—△) or IgG-C7 (□—□). The specific binding observed in the presence of pre-immune serum was arbitrarily set at 100%. Panel B: monolayers of HepG2 cells (△—△) and human fibroblasts (□—□) were incubated with ^{125}I-labeled r-apo[a] in the presence of various concentrations of ε-ACA as indicated. Binding in the absence of ε-ACA was arbitrarily set at 100%. The results represent the mean ± SEM of three separate experiments.

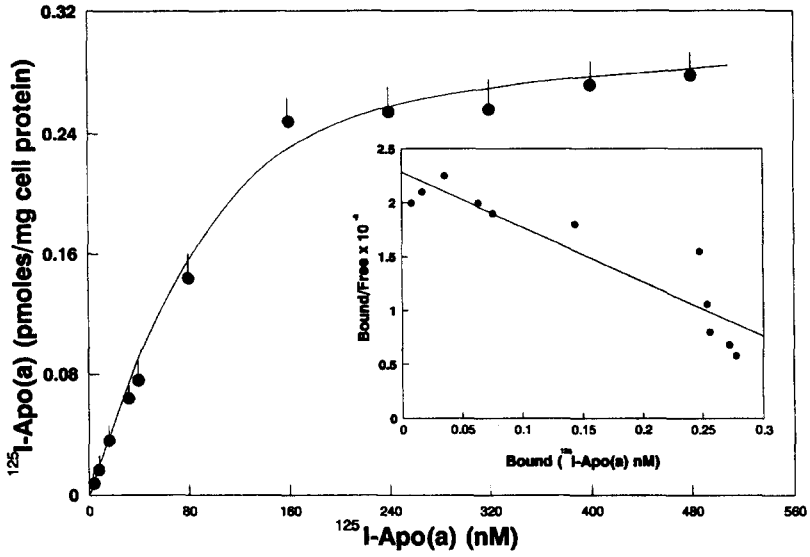

Fig. 6. Effect of heparin on the specific binding of ^{125}I-labeled r-apo[a] to HepG2 cells. Binding of ^{125}I-labeled r-apo[a] to HepG2 cells was determined after pretreatment with heparin (10 mg/ml) followed by extensive washing with PBS to remove the heparin. Scatchard transformation of the binding curve is indicated in the inset. The apparent K_d was approximately 200 nM. The results represent the mean ± SEM of three separate experiments. For ease of presentation, only mean values are shown in the Scatchard plot.

radioimmunoassay. In order to remove all apoB from the culture medium, HepG2 cells were incubated with 100 μg/ml specific anti-apoB polyclonal antibodies as previously described (36). After treatment with the anti-apoB antibodies, it was found that the binding of ^{125}I-labeled r-apo[a] to the high affinity binding sites was totally abolished. A type of saturation curve similar to that obtained after heparin pretreatment was observed in these studies, suggesting that the binding to the low affinity sites was not affected (data not shown).

Binding of ^{125}I-labeled r-apo[a] to human fibroblasts

In order to further investigate the involvement of apoB or LDL in the binding of r-apo[a] through the high affinity binding sites, binding of r-apo[a] to normal human fibroblasts (which unlike HepG2 cells do not produce endogenous apoB) was also studied. Cells were preincubated with LPDS overnight, and specific binding as a function of increasing concentrations of ^{125}I-labeled r-apo[a] was determined. The data in **Fig. 7** show that the binding curve reaches a plateau corresponding to a concentration of ~0.24 pmol r-apo[a]/mg cellular protein. Scatchard transformation of the binding curve resulted in a linear plot with a K_d of ~200 nM (i.e., similar to the low affinity binding component of HepG2 cells; Fig. 7A). Our data concerning the uptake of r-apo[a] by HepG2 cells suggested that r-apo[a] requires interaction with apoB-containing lipoproteins for internalization by the high affinity receptor component (see above). In order to assess whether fibroblasts contain a similar high affinity component, fibroblasts were incubated at 4°C with various concentrations of ^{125}I-labeled r-apo[a] in the presence of unlabeled LDL, followed by removal of the medium and incubation at 4°C for a further 3 h. Under these conditions, r-apo[a] bound to the cell surface through both high affinity (K_d = 5.2 nM) as well as low affinity (K_d = 196 nM) sites (Fig. 7B) as was observed using HepG2 cells (see Fig. 2).

The effect of LDL in the binding of r-apo[a] to the high affinity binding sites was examined using human fibroblasts derived from both a normal individual and an individual homozygous for familial hypercholesterolemia (FH). To avoid interference from the low affinity binding sites, the binding studies were carried out in the range of 0–20 nM of ^{125}I-labeled r-apo[a]. The calculated B_{max} values for normal and FH fibroblasts were 0.078 and 0.002 pmol/mg protein, respectively (**Fig. 8**). In addition, the presence of EDTA (10 mM) in the culture medium almost completely abolished the specific binding of 0–20 nM ^{125}I-labeled r-apo[a] to both types of human fibroblasts (Fig. 8).

Effects of addition of anti-LRP/α_2MR antibody, heparinase, chondroitinase ABC, or sodium chlorate on cell association of ^{125}I-labeled r-apo[a] in cultured cells

To examine whether or not the rabbit anti-LRP monoclonal antibody IgG-5D7 was able to block human LRP receptor activity, cell association of ^{125}I-labeled α_2M was determined in HepG2 cells and human fibroblasts. The amount of ^{125}I-labeled α_2M associated with HepG2 cells or fibroblasts was not affected by either lipoprotein

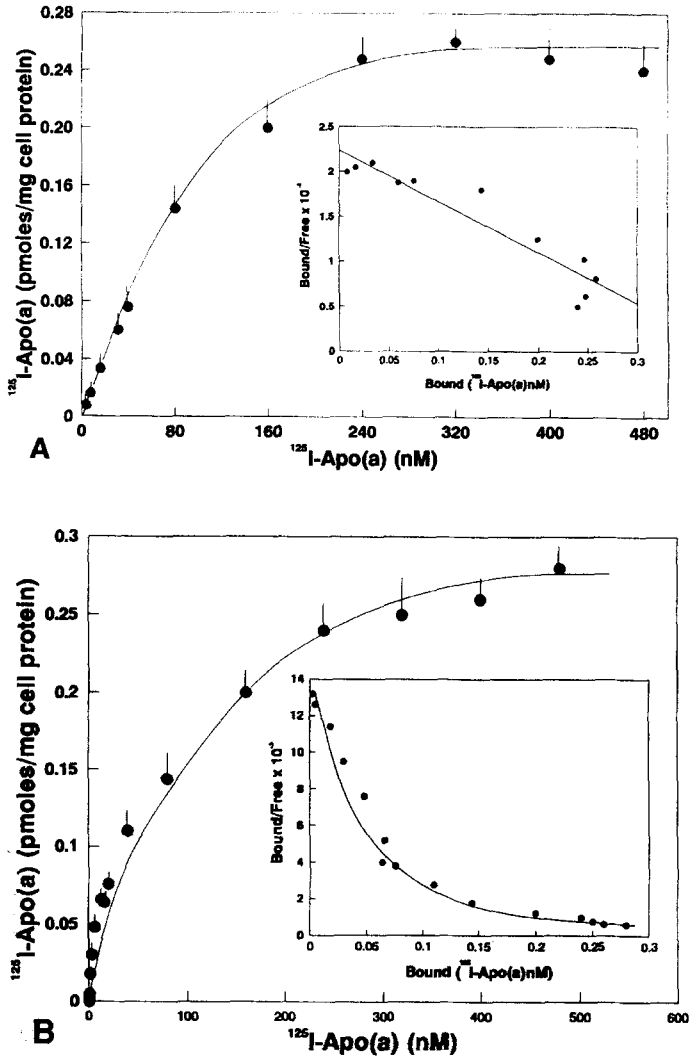

Fig. 7. Binding of ^{125}I-labeled r-apo[a] to normal human fibroblasts. (A) Experiments were performed using 90–95% confluent monolayers in 24-well tissue culture plates. A saturation curve for binding of ^{125}I-labeled r-apo[a] was determined under conditions described in Fig. 2 with increasing concentrations of ^{125}I-labeled r-apo[a] in the presence of a 50-fold molar excess of unlabeled r-apo[a]. Scatchard analysis of the binding curve is presented in the inset panel. Values are the mean ± SEM of three separate experiments. (B) Effect of LDL on binding of ^{125}I-labeled r-apo[a] to normal human fibroblasts. Binding experiments were carried out essentially as described in 7A, except in the presence of 6 nM unlabeled LDL. Similar binding results were also observed when ^{125}I-labeled r-apo[a]-LDL associated complex was used. The formation of this complex has been described in Materials and Methods. The saturation curve represents specific binding (total minus nonspecific) and is the mean ± SEM of three separate experiments. Nonspecific binding amounted to < 15% of total binding. The inset contains the Scatchard transformation of the binding curve. For ease of presentation, only the mean values are shown in the Scatchard plot.

depletion or preincubation of cells with an antibody against human albumin (**Fig. 9**). However, when the cells were preincubated with the monoclonal antibody (IgG-5D7) against the 515 kDa subunit of LRP/α_2MR, the amount of ^{125}I-labeled α_2M associated with the cells was reduced by 90%. Hence, IgG-5D7 antibody was used to investigate the involvement of the LRP receptor in the binding of r-apo[a] by HepG2 cells and human fibroblasts. Cell association studies revealed that this antibody was not able to block the specific association of r-apo[a] to the hepatoma cells or fibroblasts (**Fig. 10A and B**).

In the next series of experiments, an attempt was made to further characterize the low affinity site(s) on HepG2 cells and fibroblasts that are responsible for binding of r-apo[a]. Digestion of cells with either heparinase (5 units/ml) or chondroitinase (0.25 units/ml) for 2 h at 37°C had no significant effect on ^{125}I-labeled

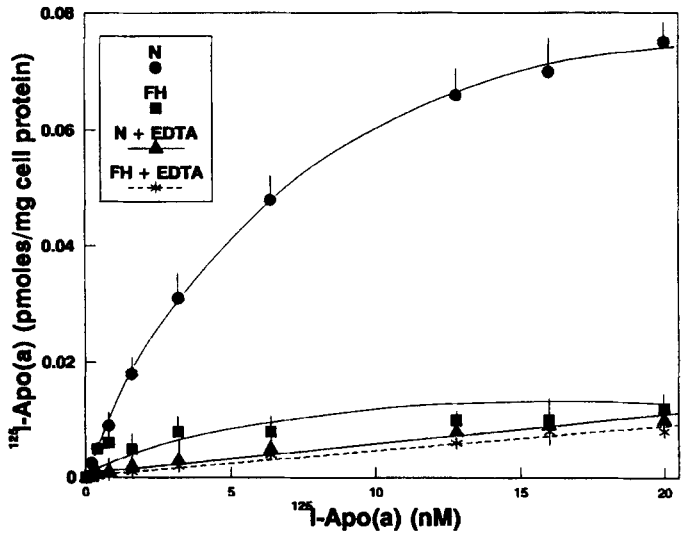

Fig. 8. Effect of EDTA on LDL-mediated ^{125}I-labeled r-apo[a] binding to human fibroblasts derived from normal individuals and FH homozygotes. Experiments were performed as described in the legend to Fig. 7. Specific binding studies of ^{125}I-labeled r-apo[a] to normal fibroblasts (N) and fibroblasts obtained from FH homozygotes was determined in the presence of 6 nM LDL. Binding experiments were also carried out in the presence of 6 nM LDL plus 10 mM EDTA using fibroblasts from both normal (N + EDTA) and homozygous FH individuals (FH + EDTA). The results are the mean ± SEM of three separate experiments.

r-apo[a] association in HepG2 cells or human fibroblasts. Furthermore, preincubation of the cells for 48 h with 30 mM sodium chlorate, an inhibitor of proteoglycan sulfation, did not affect cell association of ^{125}I-labeled r-apo[a]. However, a 50-fold molar excess of plasminogen was able to inhibit the cell association of ^{125}I-labeled r-apo[a] in both cell types by 90% (Figs. 10A and B).

Cellular uptake and degradation of ^{125}I-labeled r-apo[a]

HepG2 cells were allowed to bind ^{125}I-labeled r-apo[a] at 37°C for 6 h; the unbound ^{125}I-labeled r-apo[a] was then removed and the cells were washed extensively with PBS. To determine the amount of ^{125}I-labeled r-apo[a] bound to the cell surface, monolayers were treated with 1 ml of 0.05% trypsin as described in Materials and Methods. In this study, the radioactivity not released by trypsin corresponds to internalized r-apo[a]. Uptake of ^{125}I-labeled r-apo[a] was calculated to be 1.3 ± 0.2 pmol/mg cellular protein per 6 h, 0.9 ± 0.1 pmol/mg cell protein per 6 h, and 0.72 ± 0.1 pmol/mg cellular protein per 6 h in HepG2 cells, normal human fibroblasts, and FH fibroblasts, respectively (experiments were performed in triplicate). During a 6-h incubation at 37°C, HepG2 cells, normal fibroblasts, and FH fibroblasts degraded 0.63 ± 0.05 pmol, 0.42 ± 0.04, and 0.34 ± 0.03 pmol of ^{125}I-labeled r-apo[a]/mg cell protein, respectively.

To investigate whether degradation of ^{125}I-labeled r-apo[a] occurs in lysosomes, the effect of the lysosomotropic agent chloroquine was studied in HepG2 cells. During a 6-h incubation, chloroquine (100 μM) reduced total iodotyrosine (intracellular, cell surface, and medium accumulation) by 80%, suggesting a lysosomal pathway for degradation of internalized r-apo[a] (**Fig.**

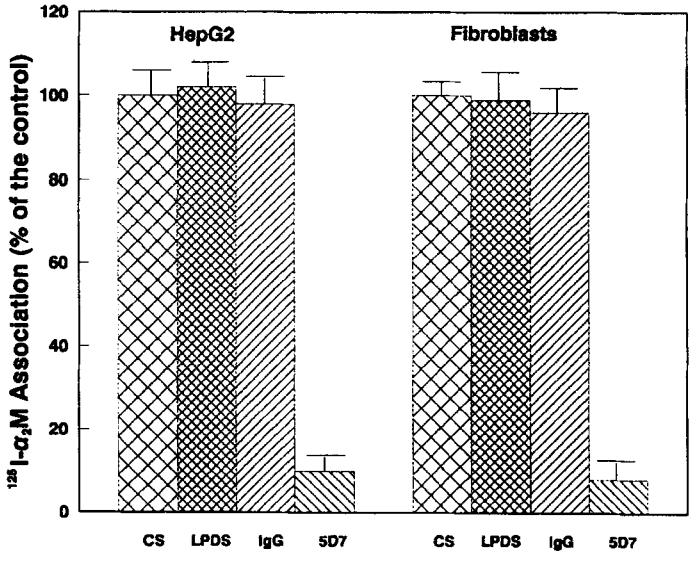

Fig. 9. Cell association of iodinated human α_2M to HepG2 cells and human fibroblasts. Cell association of ^{125}I-labeled α_2M has been described in Materials and Methods. Specific cell association was calculated by subtracting total cell association from nonspecific association. Nonspecific cell association was determined by carrying out the experiment in the presence of a 50-fold molar excess of unlabeled α_2M. Human hepatoma cells and fibroblasts were preincubated with either 10% complete serum (CS) or 10% lipoprotein-depleted serum (LPDS) or control IgG (antibody against human albumin) or the monoclonal antibody LRP/α_2MR (antibody against LRP/α_2MR). Cell association of ^{125}I-labeled α_2M in the presence of CS was arbitrarily set at 100%. Results are the mean ± SEM of three independent experiments.

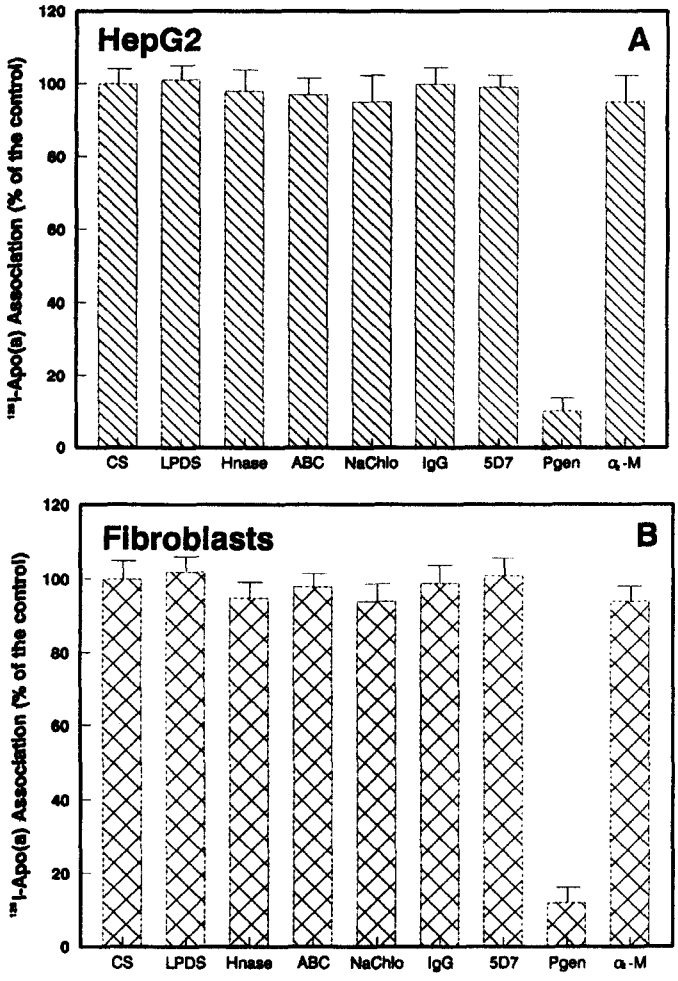

Fig. 10. Cell association of ^{125}I-labeled r-apo[a] and human hepatoma cells and fibroblasts cultured under various conditions. Cell association experiments (37°C for 3 h) and preincubation conditions were described in Materials and Methods. Specific cell association was calculated as described in the legend to Fig. 4. To avoid the interference of the high affinity site, the experiments were carried out in the presence of 10 mM EDTA, with the exception of the incubation with IgG-5D7. Panel A: monolayers of HepG2 cells were preincubated with one of the following: 10% complete serum (CS), 10% lipoprotein-depleted serum (LPDS), heparinase (Hnase), chondroitinase ABC (ABC), sodium chlorate (Na Chlo), a control antibody against human albumin (IgG), or a monoclonal antibody against LRP/α_2MR (IgG-5D7). Cell association assays were also performed in the presence of either 50-fold molar excess plasminogen (pgen) or α_2M. Panel B: cell association of ^{125}I-labeled r-apo[a] to human fibroblasts was performed as described in panel A. Cell association of ^{125}I-labeled r-apo[a] in the presence of CS was arbitrarily set at 100%. The contribution of the high affinity component during the incubation of IgG-5D7 in the absence of 10 mM EDTA has been subtracted. Results represent the mean ± SEM of three independent experiments.

11A). Furthermore, ε-ACA inhibited degradation of ^{125}I-labeled r-apo[a] by ~70% (Fig. 11A). The effect of LDL on ^{125}I-labeled r-apo[a] degradation was studied using normal human fibroblasts. In the absence of LDL, the degradation of r-apo[a] was reduced by ~20%. In contrast, degradation of r-apo[a] by FH fibroblasts was similar in the presence or absence of added LDL (Fig. 11B). As a control experiment, the degradation of ovalbumin in fibroblasts was examined. Under the above conditions, < 1% of ovalbumin was degraded (Fig. 11B).

DISCUSSION

Very little is known concerning the route of clearance of lipoprotein[a] from plasma, and the data that exist represent a number of conflicting results. According to some studies, the LDL receptor is either not involved or is involved to a very minimal extent. Other studies have indicated a role for the LDL receptor in Lp[a] clearance, in addition to a nonspecific clearance pathway (47); the magnitude of the latter pathway in vitro may vary in different cell types (15).

In the present study, we have demonstrated the existence of two classes of receptors on HepG2 cells and human fibroblasts through which a recombinant 17-kringle form of apo[a] can be internalized and degraded. The first class of receptor consists of high affinity, calcium-dependent binding sites (K_d = 10 nM), and was identified as the LDL receptor based on the ability of a monoclonal antibody specific for this receptor (IgG-C7) to block uptake and binding of iodinated r-apo[a] as well as ^{125}I-labeled LDL through these sites. Additionally, LDL effectively competed with r-apo[a] for binding to this receptor. Previously, other investigators and our laboratory have demonstrated that lipoprotein depletion of hepatoma cells and fibroblasts as well as treatment of cells with compactin and HDL results in up-regulation of the LDL receptor (41–45). In contrast, culturing the cells in the presence of either 25-hydroxy-

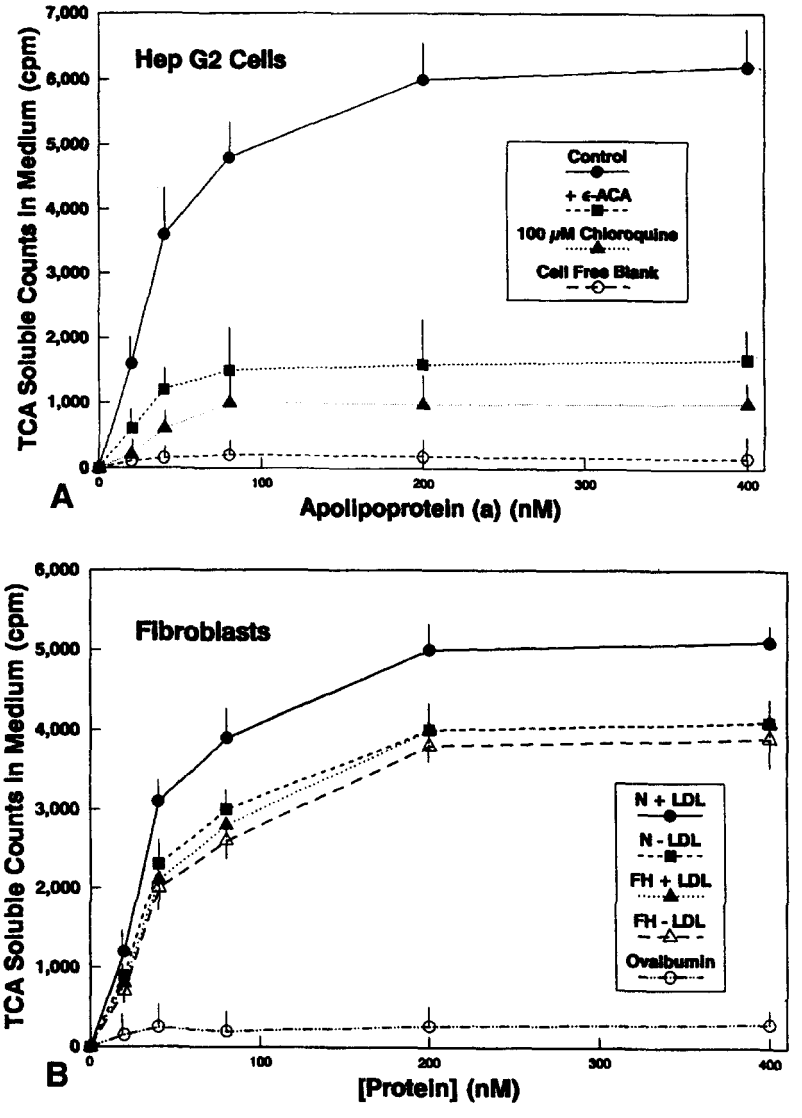

Fig. 11. Degradation of ^{125}I-labeled r-apo[a] by HepG2 cells, normal, and FH human fibroblasts. Experiments were performed as described in Materials and Methods. Degradation was measured as TCA-soluble radioactivity in the medium after incubation for 6 h at 37°C. (A) Degradation of ^{125}I-labeled r-apo[a] (specific activity = 40–60 cpm/ng protein) in HepG2 cells was carried out in the absence (●-●) or presence of either ε-ACA (10 mM) (■—■) or chloroquine (100 μM) (▲—▲). A cell free blank control experiment (○—○) is also indicated. (B) Degradation of ^{125}I-labeled r-apo[a] in normal human fibroblasts (N) was performed in the absence of LDL (■—■) or in the presence of 6 nM LDL (●-●). FH fibroblasts (FH) were also used to study the degradation of ^{125}I-labeled r-apo[a] in the absence (△—△) or in the presence (▲—▲) of 6 nM LDL. As a control, degradation of ^{125}I-labeled ovalbumin (specific activity = 100–200 cpm/ng protein) was also determined (○—○). Results are the mean ± SEM of three independent experiments.

cholesterol or the Sandoz compound 58-035 is associated with a reduction in LDL receptor levels (42, 46). The data in the present study show that in HepG2 cells, the cell association of ^{125}I-labeled r-apo[a] and ^{125}I-labeled LDL to the high affinity site parallels the up- and down-regulation of the LDL receptor level.

After heparin treatment of the cells, which removes LDL associated with the LDL receptor, binding of the r-apo[a] to this receptor was inhibited, suggesting that the r-apo[a] must be complexed with endogenous LDL produced by the HepG2 cells to form r-Lp[a] particles prior to uptake by the LDL receptor. This result was verified by incubation of the HepG2 cells with an apoB-specific polyclonal antibody, which also abolished high affinity r-apo[a] binding. The involvement of apoB in high affinity binding (i.e., the LDL receptor-mediated pathway) was further studied using human fibroblasts, which, unlike HepG2 cells, do not synthesize apoB. In

the absence of exogenous apoB, r-apo[a] bound to the cells through low affinity sites only. However, in the presence of a small amount of LDL, r-apo[a] bound to high affinity sites as well, as was observed using HepG2 cells. The identity of the high affinity receptor class on fibroblasts as the LDL receptor was demonstrated by the use of fibroblasts obtained from an individual homozygous for familial hypercholesterolemia, a disorder resulting from deficiency or a functional defect in the LDL receptor (48). Using these cells, binding of r-apo[a] to the high affinity receptor was almost totally abolished.

Our data using HepG2 cells are consistent with the recent findings of Kostner (23) which indicate that interaction of Lp[a] with these cells can be mediated through the LDL receptor by a "hitch-hiking" process. This latter pathway appears to involve binding of apo[a] to apoB-containing particles prior to their uptake by the LDL receptor. In our study, it is not clear whether disulfide linkage of apo[a] to apoB is obligatory for receptor internalization. Extracellular association studies using ^{35}S-labeled r-apo[a] and plasma containing LDL indicate that both non-covalent and covalent interactions occur between the r-apo[a] and apoB (2). The ability of both apo[a] and Lp[a] to form strong non-covalent bonds with apoB-containing lipoproteins has been clearly demonstrated in vitro (49).

We have carried out a series of experiments to identify and characterize the nature of the low affinity binding site. No significant effect of ^{125}I-labeled r-apo[a] association was observed in HepG2 cells and human fibroblasts either when the cells were depleted of heparan sulfate by heparinase treatment or when sulfation was inhibited by the addition of sodium chlorate treatment. Furthermore, removal of chondroitin sulfate by chondroitinase ABC treatment did not seem to affect r-apo[a] association with the cells. In order to avoid the possibility of regeneration of cell surface proteoglycans after the removal of heparinase, chondroitinase, or sodium chlorate during the cell association assays at 37°C, the experiments were also repeated at 4°C. After subtracting the intracellular portion of ^{125}I-labeled r-apo[a] (corresponding to uptake which occurred at 37°C), the specific binding of ^{125}I-labeled r-apo[a] to the cells under the above culturing conditions was similar at 4°C and 37°C (data not shown). Taken together, our data demonstrate a lack of involvement of cell surface glycosaminoglycans or proteoglycans in the low affinity binding component. Recently, Williams and co-workers (50) have reported that enhanced degradation of Lp[a] by lipoprotein lipase (LpL) in HepG2 cells has at least two components. One component is LDL receptor-dependent and unaffected by heparitinase digestion of the cells, and the other is LDL receptor-independent and heparitinase-sensitive. It has been proposed that LpL enhances the binding of Lp[a] or lipoproteins to cells by acting as a bridge between lipoproteins in the medium and proteoglycans on the cell surface (50). However, as supported by our data, there is no evidence at present to suggest a direct interaction between Lp[a] and cell-surface glycosaminoglycan chains.

We also have provided data to exclude the LRP/α_2MR as the low affinity site for r-apo[a]. First, it has been well established that the binding of lipoproteins to LRP/α_2MR is calcium-dependent (51). However, the low affinity site for r-apo[a] in the present study is not sensitive to EGTA treatment (see Fig. 10). Second, we have demonstrated that the monoclonal antibody IgG-5D7 raised against the ligand binding domain of the rabbit LRP/α_2MR is capable of blocking cell association of human ^{125}I-labeled α_2M to human hepatoma cells and fibroblasts (Fig. 9). We have demonstrated that this antibody (IgG-5D7) has no significant effect on cell association of r-apo[a] to the cells (Fig. 10). Finally, we performed r-apo[a] binding studies in the presence of activated α_2-macroglobulin. No competition effect was observed, which suggests that the interaction of r-apo[a] or of r-apo[a]–apoB complexes with HepG2 cells and human fibroblasts is not mediated by the LRP (see Fig. 10). Interestingly, a study by März et al. (21) suggests that the LRP may play a role in the clearance of high molecular weight Lp[a]. One explanation for this discrepancy is that their preparation of Lp[a] may contain some apoE, which could facilitate its clearance by the LRP.

The low affinity, calcium-independent binding site on HepG2 cells and human fibroblasts that was identified in our study (K_d = 200–300 nM) likely represents the plasminogen receptor. Lp[a] has been previously shown to compete with plasminogen for cellular binding sites, mediated by lysine affinity sites present in both apo[a] and plasminogen (5, 6). In the present study, we have demonstrated that plasminogen can compete with r-apo[a] for binding or cell association to the lower affinity receptor class (Figs. 4 and 10) and also that binding of r-apo[a] can be inhibited by the lysine analogue ε-ACA (Fig. 5). Additionally, we have shown that tPA (which contains two kringle domains, the second of which has been shown to be capable of lysine binding; (52)) does not compete with r-apo[a] for binding to this receptor, suggesting that the low affinity binding sites do not simply represent general lysine-rich domains present on the cell surface.

Treatment of the cells with either heparin or an apoB-specific antibody did not affect r-apo[a] binding to the low affinity receptors. Thus, our data suggest that analogous low affinity binding sites may represent an efficient route of clearance for uncomplexed apo[a] in vivo, as free apo[a] is virtually undetectable in human

plasma (53), even in patients with abetalipoproteinemia (i.e., lacking apoB) (54). The absence of uncomplexed apo[a] in plasma may be accounted for either by quantitative coupling to LDL in plasma or rapid clearance of the free apo[a] from the circulation. Our data provide a basis for the latter possibility, as plasminogen receptors are present in high copy on a wide variety of cell types.

The present studies demonstrate that degradation of r-apo[a] by HepG2 cells is inhibited by ε-ACA, indicating that uptake of r-apo[a] is necessary for degradation to occur. Furthermore, the degradation process is chloroquine-sensitive, suggesting that the proteolytic degradation takes place in lysosomes. The LDL receptor appears to be involved in the uptake and degradation of r-apo[a] only to a minor extent (Fig. 11). This portion of r-apo[a] degradation is dependent on the presence of LDL; as such, we found that uptake and degradation of r-apo[a] through this receptor did not occur in the absence of LDL. As expected, FH fibroblasts degraded ^{125}I-labeled r-apo[a] to a similar extent in the absence or presence of LDL due to the lack of functional LDL receptors on these cells. These data also imply that the plasminogen receptor is capable of binding and internalizing both free and LDL-complexed apo[a].

In a study reported by Bottalico and co-workers (55), the interaction of Lp[a] with cholesterol-loaded macrophages was reported not to involve the plasminogen receptor; they demonstrated that neither a 50-fold molar excess of Lys^{77}-plasminogen nor of the lysine analogue ε-ACA at a concentration of 6 mM inhibited ^{125}I-labeled Lp[a] binding and degradation by foam cells. In our study, we have shown that low affinity binding of r-apo[a] to HepG2 cells can be inhibited by either a 50- to 100-fold excess of plasminogen, or by 10 mM ε-ACA. The discrepancy between our results and those of Bottalico et al. (55) may be due to differences in Lp[a] and apo[a] binding and degradation by foam cells compared to HepG2 cells. Indeed, the Lp[a] receptor on foam cells appears to be absent in non-lipid-loaded macrophages (55).

In summary, our studies demonstrate the existence of two receptor classes on HepG2 cells and fibroblasts that can clear r-apo[a]: the LDL receptor which clears only r-apo[a] that is complexed to LDL, and the plasminogen receptor which can efficiently internalize and degrade free r-apo[a]. These data clearly suggest that a mechanism(s) for specific Lp[a] clearance from the circulation exists in vivo, and that the plasminogen receptor may represent a significant route of clearance for free apo[a].

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