A Chinese Hamster Ovarian Cell Line Imports Cholesterol by High Density Lipoprotein Degradation*

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Tamara A. Pagler1, Solmaz Golsabahi, Marlon Doringer, Sebastian Rhode, Gerhard J. Schütz, Margit Pavelka, Christian Wadsack, Martin Gauster, Alfred Lohninger, Hildegard Laggner, Wolfgang Strobl, and Herbert Stangl1, 2

From the 1Center for Physiology and Pathophysiology, Institute of Medical Chemistry, Medical University of Vienna, Währingerstrasse 10, A-1090 Vienna, Austria, 2Institute of Biophysics, Johannes-Kepler-University Linz, Altenbergerstrasse 69, A-4040 Linz, Austria, 3Center for Anatomy and Cell Biology, Department of Cell Biology and Ultrastructure Research, Medical University of Vienna, Schwarzenbergstrasse 17, A-1090 Vienna, Austria, and the 4Clinic of Obstetrics and Gynecology, Medical University of Graz, Auenbruggerplatz 14, A-8036 Graz, Austria

Plasma high density lipoprotein (HDL) is inversely associated with the development of atherosclerosis. HDL exerts its atheroprotective role through involvement in reverse cholesterol transport in which HDL is loaded with cholesterol at the periphery and transports its lipid load back to the liver for disposal. In this pathway, HDL is not completely dismantled but only transfers its lipids to the cell. Here we present evidence that a Chinese hamster ovarian cell line (CHO7) adapted to grow in lipoprotein-deficient media degrades HDL and concomitantly internalizes HDL-derived cholesterol. Delivery of HDL cholesterol to the cell was demonstrated by a down-regulation of cholesterol biosynthesis, an increase in total cellular cholesterol content and by stimulation of cholesterol esterification after HDL treatment. This HDL degradation pathway is distinct from the low density lipoprotein (LDL) receptor pathway but also degrades LDL. Hydroxycholesterol, a potent inhibitor of the LDL receptor pathway, downregulated LDL degradation in CHO7 cells only in part and did not down-regulate HDL degradation. Dextran sulfate released HDL bound to the cell surface of CHO7 cells, and heparin treatment released protein(s) contributing to HDL degradation. The involvement of heparan sulfate proteoglycans and lipases in this HDL degradation was further tested by two inhibitors genistein and tetrahydro lipstatin. Both blocked HDL degradation significantly. Thus, we demonstrate that CHO7 cells degrade HDL and LDL to supply themselves with cholesterol via a novel degradation pathway. Interestingly, HDL degradation with similar properties was also observed in a human placental cell line.

Plasma high density lipoprotein (HDL)2 cholesterol levels inversely correlate with the incidence of atherosclerotic cardiovascular disease. HDL cholesterol is a better predictor of coronary artery disease than low density lipoprotein (LDL) cholesterol (1). The atheroprotective effect of HDL is mainly due to its role in reverse cholesterol transport by which HDL removes excess cholesterol from peripheral tissues (2–4) and cells, including those in the artery wall (3–8), and delivers it back to the liver for disposal (9, 10). Evidence indicates that HDL also exerts antioxidative and antithrombotic effects (11–13), consistent with its atheroprotective function. In addition, HDL delivers cholesterol directly to steroidogenic tissues for steroid hormone synthesis and for storage in cholesteryl ester droplets (14–16).

The role of HDL in reverse cholesterol transport relies on the specific interaction with both peripheral and hepatic cells, but the mechanisms of cholesterol and cholesteryl ester exchange between cells and HDL are incompletely understood. Receptor-independent and receptor-dependent processes have been proposed to explain the transfer of cholesterol and cholesteryl ester between cell surfaces and HDL particles (6). In the receptor-independent model, diffusion is thought to account for both uptake of cholesteryl ester and efflux of free cholesterol. Diffusion, however, is considered to be relatively inefficient (3). Evidence for the existence of several “HDL receptors” came from the fact that HDL binds to a variety of cells with varying degrees of specificity (for reviews, see Refs. 17 and 18). Acton et al. (19) provided evidence that scavenger receptor class B, type I (SR-BI), a member of the CD36 family (20), can mediate selective uptake of HDL-derived cholesteryl ester to cells without degradation of the particle, which is termed selective cholesteryl ester uptake. In addition, a number of other receptors have been described to bind HDL: HB 1 and HB 2 (for a review, see Ref. 18), cubulin, megalin, and amnionless (21–23), class A scavenger receptors (24), CD36 (25), high density lipoprotein-binding protein (GPI-HBP1) (26), β-chain of ATP synthase (27), and several ABC transporters like ABCA1 (28–32). Little is known about the purpose and function of several of these HDL-binding proteins regarding HDL uptake and lipid delivery.

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† Supported by Austrian Science Foundation Grants J1488-GEN and P16362-B07 and OENB Fond Project 8198. To whom correspondence should be addressed: Center for Physiology and Pathophysiology, Institute of Medical Chemistry, Medical University of Vienna, Währingerstrasse 10, A-1090 Vienna, Austria. Tel.: 43-1-4277-60283; Fax: 43-1-4277-60881; E-mail: Herbert.Stangl@meduniwien.ac.at.

‡ The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; fad-BSA, fatty acid-free bovine serum albumin; PBS, phosphate-buffered saline; FCS, fetal calf serum; HLPDS, human lipoprotein-deficient serum; CHO, Chinese hamster ovarian; SR-BI, scavenger receptor class B, type I; THL, tetrahydro lipstatin; HSPG, heparan sulfate proteoglycan(s); DMEM, Dulbecco’s modified Eagle’s medium; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; Dil, 1,1’dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate.
Selective cholesteryl ester uptake mediated by SR-BI is a key mechanism in receptor-mediated delivery of HDL cholesteryl esters to liver and steroidogenic tissues. In this process, only cholesteryl esters of HDL particles but not apolipoproteins are transferred to cells (9, 10). This mechanism is distinct from the well-characterized LDL receptor pathway, in which LDL is internalized by coated pits after binding to the LDL receptor and directed to lysosomes for particle degradation (33). In both pathways, cholesterol is delivered to the endoplasmic reticulum, where excess cholesterol is esterified by acyl-CoA:cholesterol acyltransferase.

In contrast to the selective uptake of HDL-derived cholesteryl ester by SR-BI, several alternative holo-HDL uptake pathways exist. First, an apoE-independent uptake and resecretion pathway for holo-HDL has been described by several investigators (34–37). Recently, SR-BI was identified as a receptor facilitating this HDL uptake, which is followed by retroendocytosis without degradation of HDL particles (37–39). Recent studies in rodent models of genetic obesity suggest that leptin participates in the regulation of plasma HDL levels in part by stimulating holo-HDL uptake by liver cells (40, 41). The molecular details of these processes are unknown. Second, apoE-associated HDL is taken up by cells via the B/E receptors and is degraded. Interestingly, apoE recycling has been reported to occur in hepatocytes and macrophages, where a part of the apoE associated with HDL escapes degradation (42, 43). Third, the cubilin/amnionless-megalin receptor complex can mediate endocytosis of both HDL (binding to cubilin) and LDL (binding to megalin) in the proximal convoluted tubule of the kidney and the visceral yolk sac (21, 44); for review, see Ref. 23. Besides their role for the reabsorption of apoA-I in the proximal convoluted tubule (21, 23), both receptors have been reported to play a role in the delivery of nutrients like cholesterol from the maternal to the fetal side (23, 45) of the placenta. Although the clearance of apoA-I by the kidney and the uptake of apoE-containing HDL via the LDL receptor family are well established, many other facets of HDL catabolism are still incompletely understood.

The aim of the present work was to study how a line of CHO cells (CHO7) specifically adapted for continuous growth in lipoprotein-poor medium in the laboratory of Drs. Brown and Goldstein (46), survives in this medium with almost no external cholesterol. In this work, we present evidence that the CHO7 cells actively degrade HDL as well as LDL for cholesterol supply. Cholesterol delivery is demonstrated by an increase of the cellular cholesterol content leading to a down-regulation of cholesterol biosynthesis and a stimulation of cholesterol esterification after incubation with HDL. In addition, HDL associated with cells can be released by dextran sulfate, and the protein(s) responsible for this HDL degradation are in part released by heparin treatment. Furthermore, when we used the two inhibitors, genistein and tetrahydroliptatin (THL), blocking heparan sulfate proteoglycans (HSPG) or lipoprotein lipase activity, respectively, a marked reduction in HDL degradation was seen. Finally, HDL degradation with similar properties was present in a human placental cell line.

Taken together, the pathway of lipoprotein degradation and cholesterol delivery to cells described here is clearly distinct from the known HDL uptake pathways as well as from the receptor-mediated endocytosis via the LDL receptor.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Treatment**—Two Chinese hamster ovarian cell lines, CHOKI and CHO7, were used; CHO7 cells were derived from CHOKI cells through propagation in lipid-deficient serum (46). For all experiments, cells were plated in 6-well plates at a cell density of 300,000 cells/well in a 1:1 mixture of Dulbecco’s minimal essential medium (DMEM) and Ham’s F-12 medium (medium A) with 100 units/ml penicillin and 100 µg/ml streptomycin sulfate, supplemented with 5% fetal calf serum (FCS) (Invitrogen) for CHOKI or 5% human lipoprotein-deficient serum (HLPDS) for CHO7. On day 2, CHO7 cells were switched to medium A containing 5% (v/v) HLPDS, and all cells were treated with 10 µM tauparin and 100 µM melyonate for 24 h except when stated otherwise. On day 3, cells were washed with PBS and reseeded with medium A supplemented with 2 mg/ml fatty acid-free bovine serum albumin (faf-BSA; Sigma). For inhibitor treatment, cells were preincubated for 15 min before the addition of lipoproteins.

The BeWo choriocarcinoma cell line (ATCC, Manassas, VA) was cultured in F12K (Kaighn’s modification) nutrient mixture (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (hyClone) containing 100 units/ml penicillin/streptomycin and 2 mM l-glutamine at 37 °C under 5% CO2.

**Lipoprotein Preparation and Labeling**—Plasma was collected from normolipidemic volunteers, and lipoproteins were obtained by serial ultracentrifugation. LDL was recovered at a density of 1.019 g/ml, and HDL was recovered at a density of 1.21 g/ml. The HDL used did not contain any detectable apoE, as demonstrated by SDS gel electrophoresis and subsequent silver staining. The apolipoprotein part of lipoproteins was covalently labeled with either Alexa 647 (Molecular Probes, Inc., Eugene, OR) or Cy3 (Amersham Biosciences) according to the manufacturer’s description (Molecular Probes). The lipid part of HDL particles was labeled with the fluorescent phospholipid DiI (Molecular Probes). The labeling procedure included incubation of HDL, diluted in lipoprotein-deficient serum, with DiI overnight at 37 °C followed by ultracentrifugation (47). For double labeling, DiI-HDL particles were conjugated with Alexa 647. For electron microscopy, HDL particles were covalently labeled with peroxidase using a peroxidase labeling kit (Roche Applied Science) according to the manufacturer’s protocol. Lipoproteins were iodinated with sodium (125) iodide (Hartmann Analytic, Braunschweig, Germany) using the Pierce IODO-BEADS iodination reagent kit. The labeling procedure resulted in a specific activity of the lipoprotein particles of ~2000 cpm/ng. For the experiments, lipoproteins were diluted 1:10 with unlabeled lipoprotein.

**Measurement of Cholesterol Esterification**—Cells were prepared as described above. On day 3, cells were switched to media A with the addition of 2 mg/ml faf-BSA and the indicated concentrations of HDL or LDL. After 5 h, cells were pulsed with 0.2 mM [3H]oleate (~10,000 dpm/nmol; Amersham Biosciences) for 2 h and afterward washed three times with buffer A (50 mM Tris, 0.9% NaCl, and 0.2% bovine serum albumin, pH 7.4) and once with buffer B (50 mM Tris and 0.9% NaCl, pH 7.4).
Lipids were extracted twice from cell monolayers with hexane/isopropyl alcohol (3:2) and analyzed by TLC (Silica Gel G) (48). For protein analysis, the cells were dissolved in 0.1 M NaOH. Protein concentration was estimated using the Bradford Reagent (Bio-Rad). Data are presented in pmol of cholesteryl [14C]oleate/mg of cell protein.

Assay of 125I-HDL or 125I-LDL Degradation—Cells were grown and treated as described above. On day 3, cells were incubated with iodinated lipoproteins for the indicated time, media were collected, and proteins were precipitated using 50% trichloroacetic acid. To remove the free iodine, the supernatant was oxidized with hydrogen peroxide and extracted with chloroform. The amount of radioactivity in the aqueous phase was quantitated with a Cobra II -counter (PerkinElmer Life Sciences) to determine the amount of acid-soluble material formed (which is almost exclusively [125I]iodotyrosine) (48). Specific degradation, representing cell-dependent proteolysis, is expressed in ng of 125I-labeled, acid-soluble protein fragments formed per mg of total cell protein. Specific degradation was calculated as the difference between total and nonspecific degradation, estimated by using a 40-fold excess of unlabeled lipoprotein.

Measurement of Cholesterol Biosynthesis—Cells were grown and prepared as described before; on day 3, cells were incubated with increasing concentrations of HDL, LDL, or 25-hydroxycholesterol (1 μg/ml), as control, in medium A containing faf-BSA with 5 μCi of [14C]acetate (45 mCi) for 7 h. Afterward, cells were washed, and lipids were extracted and analyzed using TLC as described for cholesterol esterification. The amounts of cholesterol, cholesteryl ester, fatty acids, and triglycerides synthesized by the cells were analyzed by scintillation counting and expressed as pmol/mg of cell protein.

Determination of Cellular Cholesterol and Cholesteryl Ester Content—Cells were grown and treated as described above. After incubation with or without lipoproteins, cells were washed twice with buffer A and once with buffer B. Lipids were extracted directly on the dish using hexane/isopropyl alcohol (3:2) after the addition of the internal standards tricaprin (50 μg/dish, tridecanoyl glycerol; Sigma) and cholesteryl-heptadeccanoate (4 μg/dish; Sigma). The solvent was evaporated, and lipids were dissolved in chloroform. Determination of cholesterol and cholesteryl esters was performed by capillary gas chromatography as described by Lohninger et al. (49). The analyses were carried out on the GC 800 Top gas chromatograph (CE Instruments, Thermo Quest Italia S.p.A., Rodano, Italy) equipped with a programmed temperature vaporizer injector. A capillary glass liner was inserted into the vaporizer block of the programmed temperature vaporizer injection system. Three-meter (0.25-mm inner diameter) fused silica capillary columns with chemically bonded DB-5 (0.1-μm coating thickness; J & W Scientific, Folsom, CA) were used for all analyses. Hydrogen was used as carrier gas at 0.4 bar (8–10 ml/min flow rate) and nitrogen as the make-up gas. The flame ionization detector temperature was kept at 380 °C. Air and hydrogen flows were adjusted to give maximum detector response. Before use, the column was conditioned at 350 °C. The gas chromatographic system was calibrated with a mixture of standards. For determination of cholesterol and cholesteryl esters, the oven temperature was programmed from 180 to 278 °C at a rate of 10 °C/min and from 278 to 340 °C at a rate of 8 °C/min. During the first 2 min of each run, the oven temperature was kept at 180 °C, and the split system was turned off in an appropriate range after 45 s and returned after 120 s to enable the cholesterol and cholesteryl esters to reach the capillary column quantitatively. Data were analyzed using the Chrom-Card program. Results are expressed in μg/mg of cell protein.

HDL Retroendocytosis—Association, uptake, and resecretion of HDL particles in CHO7 and CHOK1 cells were studied as reported earlier (36, 39). Cells were seeded in 6-well plates and grown for 2 days in medium A with 5% FCS. Medium was changed to medium A containing HLPDS for 24 h. On the experimental day, cells were washed with PBS and incubated in DMEM containing 2 mg/ml faf-BSA for 1 h at 37 °C with 10 μg/ml 125I-HDL in duplicates. To measure association, medium was withdrawn, and cells were washed twice with buffer A and B and then lysed using 0.1 M NaOH. The cell lysate was analyzed for its content of radiolabel using a Cobra II γ-counter (PerkinElmer). Specific binding was calculated by subtracting cell association derived from cells incubated with a 40-fold excess of unlabeled HDL before the addition of 125I-HDL. For determination of HDL uptake, a subset of cells was washed after the initial association step and further incubated at 0 °C for 2 h with the addition of a 50-fold excess of unlabeled lipoprotein. This displacement treatment provides the removal of surface-bound HDL particles, leaving only internalized HDL with the cells. Again, media were removed, and cells were washed, lysed, and analyzed. To study the resorption of HDL particles, another subset of cells was warmed to 37 °C after the displacement procedure and incubated for 30 min with a 20-fold excess of unlabeled HDL. Subsequently, cells were treated as described above, and the amount of radiolabel remaining within the cells was determined. Cell protein was measured using the Bradford Reagent (Bio-Rad). The specific cell association, uptake, and resorption of HDL particles were calculated in ng of HDL/mg of cell protein by subtraction of unspecific binding.

Ultrasensitive Fluorescence Microscopy—CHO7 and CHOK1 cells were maintained on collagenized cover slips in 12-well dishes seeded at a cell density of 15,000 cells/well. Cells were grown for 2 days and then washed with PBS and incubated with DMEM containing 5% (v/v) HLPDS, 10 μM mevalonate, and 100 μM Mevalonate for 24 h. For the experimental procedure, cells were washed with PBS and switched to DMEM containing 2 mg/ml faf-BSA with either Alexa 647-Dil-HDL or Cy3-LDL (1 μg/ml) for 30 min. For imaging fluorescence-labeled lipoproteins, an epifluorescence microscope (Axiovert 200 TV; Zeiss, Oberkochen, Germany) was used as described previously (38). Large areas on the coverslip were recorded with a wide field scanning setup developed recently (50). Alexa 647 was excited with a 647-nm light from a krypton laser (Innova 300; Coherent, Santa Clara, CA), and the 514-nm line of an argon laser (2020 series; Spectra Physics, Mountain View, CA) was used for Cy3 and Dil excitation. Emitted fluorescence was collected using appropriate filter combinations and an oil ×100 immersion objective (Plan-Apochromat, numerical aperture = 1.45; Zeiss) and was detected by a cooled CCD camera (NTE-CCD...
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FIGURE 1. Cell association (left) and degradation (middle) of HDL (circles) and LDL (triangles) in CHO7 (closed symbols and lines) and CHOKI (open symbols, dashed lines) cells and time course of HDL association (■) and degradation (▲) in CHO7 cells (right). Cells were grown for 2 days as described under "Experimental Procedures," and cholesterol was depleted using 5% HLPDS, 10 μM compactin, and 100 μM mevalonate for 24 h. On day 3, cells were incubated for 5 h with increasing concentrations of 125I-HDL or 125I-LDL or for time course experiments for the indicated times with 10 μg of 125I-HDL/ml. Afterward, media were collected. For association, cells were washed twice with buffer A and B, lysed, and analyzed for their 125I content. For lipoprotein degradation, the medium was subjected to trichloroacetic acid precipitation, oxidization, and chloroform extraction. The amount of radioactivity in the aqueous phase was quantitated on a γ-counter. Data points represent the mean of triplicate values. Note the similarities of both cell lines in association but an almost linear increase in HDL degradation just in the CHO7 cells.

CHO7 cells exhibited a dose-dependent degradation of HDL particles (●) that exceeded the LDL degradation. The degradation of HDL in the CHO7 cells was in the same range as the degradation of LDL by the CHOKI cells. In contrast to LDL degradation, HDL degradation in the CHO7 cells did not reach saturation with 20 μg of HDL/ml as indicated by almost a linear slope. Consequently, we assessed the time dependence of HDL association in CHO7 cells (Fig. 1, right). The addition of 10 μg of 125I-HDL/ml resulted in a small and distinct amount of cell association in CHO7 cells (■), reaching saturation after several hours. HDL degradation increased steadily with time, almost reaching a plateau after 7 h of incubation (▲). In summary, HDL degradation exceeds cell association severalfold in CHO7 cells. Thus, the CHO7 cell line shows an unusual phenomenon, namely the degradation of intact HDL particles. This may explain the survival of CHO7 cells in HLPDS medium if this HDL degradation leads to a concomitant uptake of cholesterol.

HDL Degradation Leads to a Dramatic Increase in Cholesterol Esterification in CHO7 Cells—Therefore, we tested if the large increase in HDL degradation in CHO7 cells leads to a concomitant uptake of the cholesterol moiety derived from HDL. For this purpose, we simultaneously measured HDL degradation and stimulation of cholesterol esterification (Fig. 2). The protein moiety of HDL as well as of LDL was labeled with 125I, and cells were incubated with increasing concentrations of radiolabeled lipoproteins for 5 h. Newly synthesized cholesteryl esters were estimated by incubation with [14C]oleate for additional 2 h. As expected, CHO7 cells showed dose-dependent degradation of HDL particles (●) and also LDL particles (▲) (Fig. 2, upper left), reaching almost a plateau at higher lipoprotein concentrations (100 μg/ml). Concomitantly with the HDL degradation, a substantial increase in cholesterol esterification was observed (Fig. 2, lower left, ○). This indicates that CHO7 cells are able to take up cholesterol from HDL particles via degradation. Similarly, stimulation of esterification was seen after incubation with LDL (▲). The degradation of HDL was not seen in the parental cell line CHOKI (second column) and in a human fibroblast cell line called 398 (rightmost column), which were used as controls, indicating that the degradation of HDL was cell line-specific. Stimulation of triglyceride synthesis after incubation with [14C]oleate remained unchanged with both HDL and LDL in CHO7 and CHOKI cells (data not shown), indicating that the differences in the two cell lines are confined to the cholesterol metabolism. To assess the integrity of the LDL and HDL preparations used in the experiments, a cell line without any functional LDL receptor (ldlA7) and ldlA7 cells overexpressing SR-BI (ldlA7-SRBI cells) were used. Almost no degradation of HDL and LDL was seen in ldlA7 cells. Therefore,
almost no cholesterol was delivered to the ldlA7 cells by these two lipoproteins, indicated by the absence of stimulation of cholesterol esterification. This indicates that the particles used were not degraded in an unspecific way. There was some degradation of LDL in ldlA7-SRBI cells due to the overexpression of SR-BI (middle), which was reported previously (52). Both HDL and LDL led to the known stimulation of cholesterol esterification mediated by SR-BI in ldlA7-SRBI cells (52), again indicating that both lipoprotein preparations were intact.

Taken together, our data clearly demonstrate that CHO7 cells take up cholesterol from degraded HDL particles and transport the released cholesterol to the endoplasmic reticulum, where excess cholesterol is esterified.

CHO7 Cells Can Degrade both HDL and LDL via a Mechanism Distinct from the LDL Receptor Pathway—HDL degradation in CHO7 cells is not completely blocked by the addition of 50 μM chloroquine (●) (Fig. 2, upper left), a weak base that raises the pH of acidic compartments. Interestingly, LDL degradation is not completely blocked by chloroquine in CHO7 cells either (▲). In CHOKI cells, however, LDL degradation is, as expected, completely down-regulated. Since degradation of LDL via the LDL receptor is known to be completely blocked by chloroquine (53) (compare CHOKI), these data indicate that the degradation pathway described here for HDL also extends to LDL in CHO7 cells. Cholesterol esterification was induced by both LDL and HDL in CHO7 cells and, in contrast to lipoprotein degradation, was decreased dramatically by chloroquine treatment (Fig. 2, lower left). In CHOKI and 398 cells, LDL-dependent stimulation of esterification via the LDL recep-

tor uptake pathway was shut down completely by chloroquine (second and right panels, respectively) as described previously (53). Similarly, SR-BI-dependent stimulation of cholesterol esterification is blocked completely by chloroquine treatment in ldlA7-SRBI cells (middle) (52). Taken together, these data indicate that the pathway of HDL and LDL degradation in the CHO7 cells does not occur via the lysosomal pathway. However, the transport of the cholesterol transferred to the cells, by both the LDL receptor pathway and this alternative lipoprotein degradation pathway, converge on an acidic endosomal compartment on their transit to the endoplasmic reticulum.

To ensure that chloroquine did not directly inhibit acyl-CoA:cholesterol acyltransferase activity in our experiments, stimulation of 25-hydroxycholesterol esterification was assessed. 25-Hydroxycholesterol freely passes membranes and mimics cholesterol loading, thereby suppressing the SREBP pathway and its target genes. Chloroquine treatment did not inhibit the dose-dependent stimulation of 25-hydroxycholesterol esterification (data not shown). In addition, we applied a potent inhibitor of the acyl-CoA:cholesterol acyltransferase enzyme, Sandoz 58.035, to block acyl-CoA:cholesterol acyltransferase action. Both HDL- and LDL-induced cholesterol esterification was shut down almost completely after treatment with Sandoz 58.035, indicating an intact acyl-CoA:cholesterol acyltransferase enzyme (data not shown).

Incubation with a mixture of HDL and LDL in CHO7 cells resulted in a similar dose-dependent stimulation of cholesterol esterification and lipoprotein degradation as seen when both lipoproteins were added separately (data not shown). Thus, the
effects were nonsaturated and not synergistic but additive. From these data, we conclude that the CHO7 cells have induced certain gene(s) that enable them to take up cholesterol for survival at very low media concentrations of cholesterol via a hitherto undescribed degradation pathway.

25-Hydroxycholesterol Does Not Shut Down HDL and LDL Degradation in CHO7 Cells—Interestingly, CHO7 cells survive 25-hydroxycholesterol treatment, which shuts down cholesterol biosynthesis and the SREBP pathway in most cells, substantially longer than CHOKI cells (data not shown). The HDL degradation pathway described here could be responsible for this prolonged survival. To examine this hypothesis, we analyzed if this alternative HDL degradation pathway proceeds in the presence of 25-hydroxycholesterol (Fig. 3). In control CHOKI cells (dashed lines), degradation of LDL was almost completely blocked by 25-hydroxycholesterol (right), whereas no degradation of HDL occurred (triangles, left). In CHO7 cells (circles, closed lines), however, HDL degradation was not altered by 25-hydroxycholesterol treatment at all, whereas degradation of LDL was only partially decreased. The shape of the LDL degradation curve changed to a linear pattern in the presence of 25-hydroxycholesterol (right). These data clearly show that LDL degradation seen in CHO7 cells consists of two pathways: a poorly saturable degradation pathway and a saturable one via the LDL receptor. Thus, the novel lipoprotein degradation pathway allows the cells to survive 25-hydroxycholesterol treatment longer because lipoprotein degradation is not blocked by 25-hydroxycholesterol itself. This also demonstrates that HDL degradation is not influenced by the cholesterol status of CHO7 cells.

Cholesterol Biosynthesis Is Down-regulated after Lipoprotein Feeding in CHO7 Cells—Next, we assessed if the delivery of cholesterol via this alternative lipoprotein degradation pathway leads to a down-regulation of cholesterol biosynthesis (Fig. 4). After induction of cholesterol biosynthesis by cholesterol depletion using compactin and HLPS overnight, the cells were incubated with [14C]acetate and either LDL or HDL for 7 h. To assess the ability of the lipoproteins to inhibit cholesterol biosynthesis, the amounts of newly synthesized cholesterol and cholesteryl ester were measured by TLC (Fig. 4). 25-Hydroxycholesterol was used as a control for the down-regulation of the cholesterol biosynthetic pathway. A substantial difference in the total amount of cholesterol synthesized was seen between CHO7 and CHOKI cells, 7660 ± 1314 pmol/mg of cell protein and 13,780 ± 3320 pmol/mg of cell protein, p < 0.05, respectively. Unexpectedly, CHOKI cells showed an almost 2-fold higher cholesterol biosynthesis rate compared with CHO7 cells (compare untreated CHO7 with CHOKI controls) (Fig. 4). Cholesterol biosynthesis was down-regulated by HDL as well as LDL in CHO7 cells (gray bars, left). Although even higher doses of HDL hardly induced storage of newly synthesized cholesterol as esters, LDL induced a dose-dependent increase in the cholesteryl ester moiety (open bars). In CHOKI cells, a down-regulation of cholesterol biosynthesis was seen both using LDL and HDL. Similar results were obtained in experiments where cells were incubated overnight (data not shown). Both biosynthesis of free fatty acids and triglycerides were unchanged with the treatment regimen (data not shown). However, like for cholesterol biosynthesis, CHOKI cells exhibit in general a 2–3-fold higher synthesis rate of both free fatty acids and triglyceride compared with CHO7 cells (data not shown), indicating that CHO7 cells have a different metabolic state. These observations show that CHO7 cells must meet their cholesterol need to a higher degree from extracellular sources than CHOKI cells because CHO7 cells exhibit a much lower cholesterol biosynthesis rate.

The cholesterol biosynthesis data were confirmed by results obtained on the response of the 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) protein, the rate-limiting enzyme in cholesterol biosynthesis, to lipoprotein-mediated cholesterol uptake. HMG-CoA reductase protein was down-regulated in CHO7 cells by LDL as well as by high amounts of HDL as demonstrated by Western blotting (not shown). The well known effect of LDL on the protein levels of HMG-CoA reductase was seen in the control CHOKI cells, whereas even high concentrations of HDL did not affect the protein levels of this enzyme in CHOKI cells (not shown). In summary, these results suggest that, in contrast to CHOKI cells, the CHO7 cell line is able to deliver both HDL- and LDL-de-
CHO7 cells decreased by about 20%. A similar but somewhat lower decrease was observed in CHOKI cells. After cholesterol depletion, the cholesterol content in the CHO7 cells dropped to almost undetectable levels and remained unchanged in CHOKI cells. LDL incubation led to a dose-dependent increase in the total cholesterol content of both CHO7 and CHOKI cells. Starved CHO7 cells showed similar cholesterol efflux; however, CHO7 cells are capable of increasing their total cholesterol content after LDL incubation, whereas CHOKI cells do not. This suggests that CHO7 cells have a more efficient way to supply themselves with external cholesterol than CHOKI cells. HDL leads to a similar replenishment of the total cholesterol content after HDL feeding, although their rate of cholesterol synthesis is higher than that of CHOKI cells.

CHO7 Cells Are Able to Replenish Their Cholesterol Stores from HDL after Cholesterol Depletion—To substantiate our data, we tested if CHO7 cells are able to refill their cholesterol stores from lipoprotein-derived cholesterol after cholesterol depletion using direct analysis of their cellular cholesterol content by gas chromatography (Fig. 5). The cholesterol (top) and cholesteryl ester (middle) content of CHO7 cells incubated with 50 µg/ml HDL (closed bars) or LDL (hatched bars) was compared with that of the parental cell line CHOKI. After cholesterol depletion (open bars), the cholesterol content in the CHO7 cells decreased by about 20%. A similar but somewhat higher drop was seen in CHOKI cells (Fig. 5, top; gray versus open bars). After starvation, the cholesteryl ester content dropped to almost undetectable levels in CHO7 cells and to very low levels in CHOKI cells (Fig. 5, middle; gray versus open bars). Incubation with 50 µg/ml HDL for 7 h filled the cholesterol stores of the CHO7 but not in CHOKI cells (Fig. 5, top; open versus closed bars). The total cholesterol content of the CHO7 cells increased by ~2-fold after HDL feeding, whereas the total cholesterol levels of the CHOKI cells remained unchanged (Fig. 5, bottom). By contrast, both CHO7 and CHOKI cells were able to replenish their cholesterol stores after incubation with 50 µg/ml LDL for 7 h (hatched bars). The HDL-induced increase in the cholesterol content in CHO7 cells was dose-dependent (Fig. 6). The total cholesterol content of CHO7 cells (○, left) increased, whereas that of the CHOKI cells (△) remained almost unchanged. Again, LDL incubation led to a dose-dependent increase in the total cholesterol content of both CHO7 and CHOKI cells (Fig. 6, right). To check if cholesterol release was altered in CHO7 cells, we measured cholesterol efflux in CHO7 and CHOKI cells. Therefore, cells were trace-labeled with [3H]cholesterol overnight and then incubated with 10 µg of HDL/ml for 5 h. Afterward, the media were analyzed for the release of [3H]cholesterol. Both CHO7 and CHOKI cells showed similar cholesterol efflux: ~20 pmol of cholesterol/mg of cell protein was transferred to the media within 5 h. Taken together, CHO7 cells exhibit a more efficient way to supply themselves with external cholesterol than CHOKI cells. HDL leads to a similar replenishment of the total cellular cholesterol content as LDL in CHO7 cells. Starved CHOKI cells are not capable of increasing their total cholesterol content after HDL feeding, although their rate of cholesterol synthesis is higher than for CHO7 cells.

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FIGURE 5. Analysis of cholesterol and cholesteryl ester content in CHO7 and CHOKI cells. Both cell lines were grown in medium A with 5% FCS. On day 2, one set of cells was harvested (gray bars), parallel sets of plates were cholesterol-depleted using 5% HLPDS, 10 µM compactin, and 100 µM mevastatin for 24 h. On day 3, another set of cells was harvested (open bars). The other two subsets were further incubated either with 50 µg/ml HDL (black bars) or LDL (hatched bars) in medium A containing faf-BSA for 7 h. Afterward, the lipids were extracted and quantified using gas chromatography as described under “Experimental Procedures.” Note that HDL replenishes the cholesterol and cholesteryl ester pool after starvation only in CHO7 cells (open and black bars).

FIGURE 6. Dose response of total cholesterol content in CHO7 (circles) and CHOKI (triangles) cells to HDL (left) and LDL (right) treatment. Cells were grown and treated as described for Fig. 1. On day 3, cells were incubated with increasing concentrations of HDL or LDL for 7 h. Then cells were washed, lysed, and analyzed for their total cholesterol content as described in Fig. 5. Note the increase of total cholesterol in response to HDL feeding in CHO7 cells.
increased 5 times). Similarly, HDL lipid-DiI showed a more intense staining compared with CHOKI (second row). CHO7 cells exhibit a much higher amount of HDL binding at the cell membrane than CHOKI cells, as indicated by the bright staining (upper left). The signal for endocytosed HDL particles, however, did not vary significantly between these two cell lines. Panel A shows a magnification of a single CHO7 cell with an intense signal of HDL protein at the cell membrane area. In contrast, the control cell line CHOKI exhibits a punctuate staining pattern for HDL protein. Higher magnification (C) shows binding and uptake of HDL particles in CHOKI cells (note that the intensity scaling is increased 8 times over A). DiI fluorescence was detected in the periphery of CHO7 cells indicating lipid uptake. The DiI staining pattern correlated with the protein staining. There was colocalization of the protein and the lipid signal in the periphery of CHO7 cells. Furthermore, DiI signals could also be clearly observed within the cells in CHO7 as well as CHOKI cells (compare B and D). LDL also showed a brighter staining pattern in CHO7 cells than in CHOKI cells (bottom).

To further analyze the HDL uptake pattern, electron microscopy was performed. HDL was visualized by covalent linkage to peroxidase and subsequent staining by DAB (data not shown). Both CHO7 and CHOKI cells showed few endosomal compartments reactive for internalized HDL peroxidase. Using fluorescence microscopy, we observed larger amounts of HDL bound to the cell surface of CHO7 cells than CHOKI cells and also HDL particles internalized; however, this was not seen by electron microscopy. Therefore, we tested if HDL particle association, uptake, or resecretion were altered in CHO7 cells using a well established procedure to assess lipoprotein retroendocytosis (36, 39) (Fig. 8). As expected, CHO7 cells showed higher HDL degradation (390 ng of HDL/mg of cell protein; data not shown) than CHOKI cells (8 ng/mg) during the 1-h incubation time of the association experiment. Nevertheless, despite of higher HDL degradation, association of HDL in CHO7 was quite similar compared with CHOKI cells. This is in contrast to the increased surface binding of HDL seen in fluorescence microscopy. Although cell surface binding seen with fluorescent images represents total association, the calculated association presented in the retroendocytosis experiment represents cell association after several washing steps and the subtraction of association seen with a 40-fold excess of unlabeled HDL. This could explain the apparent discrepancy. Therefore, we tested if the HDL is loosely attached to the CHO7 cells and can be released by the extensive washing steps used. Indeed, about two-thirds of the 125I-HDL associated with these cells is removed by washing with PBS in our experiments (data not shown). This indicates that the HDL particles observed at the cell surface of CHO7 cells by fluorescence microscopy are loosely attached.
Next, uptake of holo-HDL was assessed by removing cell surface-bound \( {\text{\textsuperscript{125}}\text{I}} \)-HDL using a 50-fold excess of unlabeled HDL at \( 0^\circ\text{C} \) (Fig. 8). CHO7 cells showed a slight although not significant increase in the amount of internalized HDL, and no significant differences in HDL secretion were seen. Degradation of HDL was also seen during displacement (4 ng/mg, per 2 h) and chase (2 ng/mg, per 20 min) in CHO7 cells although to a much lower extent compared with association. Taken together, CHO7 cells exhibit similar endocytosis and exocytosis of HDL compared with CHOKI. This shows that HDL degradation in CHO7 cells is largely dependent on binding to the cell surface but not on endocytosis.

To determine whether HDL degradation increases when HDL association is increased, we used glyburide, a substance known to increase cell association of lipoproteins but to decrease selective cholesteryl ester uptake (54, 55). After glyburide treatment, HDL cell association was increased in CHO7 cells, but degradation was unaltered (data not shown). These data indicate that holo-HDL particle uptake mediated by SR-BI does not seem to be the mechanism by which CHO7 cells release HDL from HDL particles. Lipoproteins binding to the apoB,E receptors are released by negatively and positively charged molecules, such as dextran sulfate, heparin, and others (56, 57), whereas binding to scavenger receptors is sensitive to dextran sulfate and other polyamions but not heparin (58). To analyze if dextran sulfate is able to release HDL bound to the cell surface, CHO7 cells were incubated with increasing concentrations of \( {\text{\textsuperscript{125}}\text{I}} \)-HDL for 7 h, rinsed, and subsequently washed with dextran sulfate (4 mg/ml in 50 mM NaCl, 10 mM Hepes, pH 7.4) for 60 min at \( 0^\circ\text{C} \). Dextran sulfate is known to release cell surface LDL bound to the LDL receptor (56). Dextran sulfate release of HDL (Fig. 9) was only seen in CHO7 (solid lines) but not in CHOKI (dashed lines) cells. 25-Hydroxycholesterol did not alter this effect (closed symbols). These data indicate that CHO7 cells can bind and release a detectable amount of HDL, whereas CHOKI cells did not exhibit any response to dextran sulfate incubation at all.

In addition, we asked if a protein(s), secreted by the cells or loosely attached to the cell surface, mediates the degradation of HDL by CHO7 cells. First we applied conditioned media derived from CHO7 cells to the degradation assay. Neither CHO7 nor CHOKI cells showed significant changes of HDL degradation after incubation with \( {\text{\textsuperscript{125}}\text{I}} \)-HDL in conditioned medium derived from CHO7 cells (data not shown). CHO7 and CHOKI controls degraded 990 ± 190 ng of HDL/mg of cell protein and 21 ± 18 ng of HDL/mg of cell protein, respectively. Conditioned medium was centrifuged before use to remove detached cells. Heat inactivation or the addition of protease inhibitor mixture did not alter HDL degradation. Second, CHO7 cells detached from the plate using trypsin showed the known degradation of HDL, whereas the supernatant showed no specific lipoprotein degradation. These data indicate that the protein(s) responsible for the HDL degradation is neither loosely connected to the cell surface of the CHO7 cells nor secreted actively to the media.

To further characterize the surface binding of the protein(s) mediating HDL degradation in CHO7 cells, we used heparin to disrupt the attachment of heparin sulfate-bound protein(s) to the cell surface. After incubating CHO7 cells with heparin for 30 min, the conditioned media from heparin-treated cells were analyzed for their ability to degrade HDL. For this purpose, heparin media were incubated with 10 \( \mu \text{g/mg} \) of \( {\text{\textsuperscript{125}}\text{I}} \)-HDL/ml for 30 min. A dose-dependent increase in HDL degradation was seen (Fig. 10). Incubation of conditioned media derived from heparin incubation of CHOKI cells and of media complemented with heparin only but without incubation on cells did
HDL Degradation in CHO Cells

not show any specific degradation of HDL (data not shown). After heparin incubation, the CHO7 cells showed a decrease in HDL degradation (data not shown). Thus, proteins degrading HDL can be released from CHO7 cells by heparin.

HDL Degradation Is Decreased by an HSPG Pathway Inhibitor and a Lipase Inhibitor—The results gained through the heparin experiments point to an involvement of HSPG or of a lipase associated with HSPG. To test this, we applied genistein, a substance that blocks HSPG-mediated pathways for ligand internalization (59), and THL, known to inhibit lipoprotein lipase activity (60) (Fig. 11). CHO7 cells were preincubated for 15 min with one of the inhibitors before 10 μg of 125I-HDL/ml was added for 5 h. Treatment of CHO7 cells with genistein (400 μM) decreased HDL degradation by 70% versus untreated control (p < 0.001, n = 4). Furthermore, incubation of CHO7 cells with THL (25 μg/ml) showed a decrease in HDL degradation by 60% versus untreated control (p < 0.001, n = 4).

These results further substantiate that proteoglycans are involved in the novel HDL degradation mechanism. Furthermore, lipoprotein lipase also seems to play a role, since inhibition of its catalytic activity markedly reduced HDL degradation.

HDL Degradation Occurs in a Human Trophoblast-like Cell Line—Finally, we wanted to test if the unusual phenomenon of HDL degradation exists in differentiated cells. Since Wadsack et al. (61) recently described degradation of HDL in placental trophoblast cells, we used the placental trophoblast-like BeWo cell line. BeWo cells showed a significant amount of HDL degradation (181 ± 87 ng of HDL/mg of cell protein, n = 3) (see Fig. 12). HDL degradation in the BeWo cells was, like degradation in CHO7 cells, substantially higher than in CHO1 cells (~20 ng of HDL/mg of cell protein). The presence of HDL degradation in the human placental trophoblast cell line indicates that HDL degradation may occur in the human placenta. In order to determine whether the mechanism of HDL degradation is similar in both CHO7 and BeWo cells, we studied the effect of inhibitors used on HDL degradation for CHO7 cells in BeWo cells. First, we used 25-hydroxycholesterol, which did not alter HDL degradation in CHO7 cells (Fig. 3). In BeWo cells, HDL degradation decreased to 72 ± 39 ng of HDL/mg of cell protein but did not reach significance (p = 0.087; Fig. 12). Although HDL degradation in BeWo cells showed some response to 25-hydroxycholesterol, LDL degradation is normally completely shut down by 25-hydroxycholesterol. The weak response of HDL degradation to 25-hydroxycholesterol might imply that the degradation mechanisms are similar both in CHO7 and BeWo cells.

Second, to gain further insight into the similarities of HDL degradation, genistein and THL were used (Fig. 12). BeWo cells were treated with either genistein (400 μM) or THL (25 μg/ml), as described for CHO7 cells. Inhibitor treatment did reduce HDL degradation in both cells. Whereas genistein lowered HDL degradation by 37%, THL inhibition showed a 67% reduction. Inhibition of HDL degradation, however, did not reach significance for either genistein (p = 0.347) or THL (p = 0.071). Thus, in both cell models, HDL degradation decreased after genistein and THL treatment; however, the magnitude of the response was different.

Taken together, we present here a CHO7 cell line capable of degrading HDL with concomitant delivery of the cholesterol to the cells. HDL degradation is not confined to this specific CHO7 cell line but also occurs at least in a placental trophoblast-like cell line, namely the BeWo cells.

DISCUSSION

Biogenesis of HDL has been studied extensively (for details, see a recent review (62)), whereas the dismantling of circulating HDL particles has not been the focus of much research. In this study, we describe degradation of intact HDL particles by CHO7 cells (Fig. 1) and the concomitant delivery of cholesterol to the cell. These CHO7 cells were adapted to grow under low cholesterol concentrations. The phenomenon of lipoprotein degradation and lipid delivery described here is distinct from the well characterized receptor-mediated endocytosis of LDL via LDL receptor (33), which also mediates the uptake of apoE-containing HDL particles. In the process of HDL degradation by CHO7 cells, cholesterol is taken up as demonstrated by stimulation of cholesterol esterification (Fig. 2), down-regulation of cholesterol biosynthesis (Fig. 4), and replenishment of cellular cholesterol after starvation (Figs. 5 and 6). This lipoprotein degradation mechanism seems to be responsible for the prolonged survival of CHO7 cells in states of cholesterol depletion.

Interestingly, the HDL degradation pathway in CHO7 cells is not subject to feedback regulation by cellular cholesterol, at least when using the substitute 25-hydroxycholesterol. Based on this lack of regulation by the cholesterol status of the cells, one can speculate that this mechanism of lipoprotein degradation not only serves to supply the cell with cholesterol but might also be important for removing HDL or even modified or oxidized lipoproteins from the circulation.

Not only HDL but also LDL and probably modified lipoproteins (as observed for acetylated LDL; data not shown) are degraded rapidly in CHO7 cells via this alternative mechanism. LDL degradation was only partly down-regulated by 25-hydroxycholesterol in CHO7 cells. This indicates that LDL can be
degraded by two pathways in this cell line: by the alternative degradation mechanism described here and by the classical LDL receptor-mediated endocytosis.

Our data suggest that the lipoprotein degradation in CHO7 cells differs not only from LDL receptor-mediated endocytosis but also from other pathways of HDL metabolism that have been characterized so far; neither retroendocytosis (Fig. 8) nor increased expression of receptor proteins like SR-BI and LDL receptor are responsible for the HDL degradation and stimulation of esterification observed in CHO7 cells. SR-BI and LDL receptor protein levels were unaltered as judged by Western blot analysis (data not shown). Moreover, overexpression of SR-BI in cell culture only leads to a modest increase in HDL degradation (compare Fig. 2 and Ref. 52). Therefore, it is not likely that a mutation in SR-BI would lead to the large increase in HDL degradation seen in CHO7 cells. This is in line with our data indicating that gliuryride treatment, known to increase HDL binding to SR-BI, did not lead to significant changes in HDL degradation by CHO7 cells.

HDL degradation in CHO7 cells also appears to be distinct from apoA-I and apoA-II catabolism recently described in the liver of ob/ob mice (41). This study showed that leptin ablation resulted in elevated plasma HDL cholesterol and HDL apolipoprotein levels, probably due to a decreased turnover in the liver. Using hepatocytes from ob/ob mice, the same authors showed that these hepatocytes exhibited a decreased HDL association and degradation, which could be reversed by leptin treatment (40). Even in wild-type mice, a reduction in HDL cholesterol and apoprotein levels was seen after leptin treatment (41). This impaired catabolism of HDL apolipoproteins may be due to an as yet uncharacterized receptor mediating HDL holo particle uptake and degradation. In contrast, the degradation of HDL particles by CHO7 cells characterized in this study occurs mainly at the cell surface. Therefore, it is not likely that the holo-HDL particle uptake described in ob/ob mice is similar to the degradation seen in the CHO7 cells.

Recently, the β chain of ATP synthase was described to bind apoA-I with high affinity and mediate HDL endocytosis (27). Since this receptor was reported to bind only HDL but not LDL, it is not a good candidate for the degradation observed in CHO7 cells.

Additionally, the megalin, cubilin, and amnionless proteins were reported to be responsible for the endocytosis and subsequent degradation of HDL in placenta, yolk sac, ileum, and kidney (23, 63). Therefore, this receptor complex could be another candidate for the alternative degradation pathway seen in CHO7 cells. Interestingly, both cubilin and megalin expression are not altered by administration of 25-hydroxycholesterol in rat yolk sac cells (63), which is similar to the effects seen in our CHO7 cell system. However, HDL degradation in CHO7 cells, unlike cubilin-mediated HDL degradation, appears to proceed at the cell surface. Moreover, cubilin protein expression was unchanged in CHO7 cells (data not shown). Nevertheless, further experiments will be needed to determine if these receptors (e.g. by a mutation in the receptor) or other HDL binding proteins are involved in degradation of HDL by CHO7 cells.

The alternative HDL degradation pathway of CHO7 cells is not mediated by apoE. ApoE is a key mediator of internalization of lipoproteins by serving as a ligand of the LDL receptor (64, 65). Furthermore, most internalized lipoproteins mediated by apoE-receptor interaction are degraded, whereas a part of the apoE can escape degradation in hepatocytes (66). Degradation of apoE does not play an important role in the degradation described here: 1) the HDL used was checked for the presence of apoE via silver stain, and no band was detected at the area of ~35 kDa; and 2) HDL degradation was not seen in control CHO/KI cells that do also express the LDL receptor family at a normal level and should show degradation of apoE containing lipoproteins.

The HDL degradation pathway of CHO7 cells seems to be similar to the degradation of HDL in trophoblast cells, which was recently described by Wadsack et al. (61) (see also Fig. 12). In first trimester trophoblasts, degradation of HDL was even higher than HDL cell association (61), resembling the results presented in Fig. 1. This indicates that trophoblasts are capable of dismantling high amounts of HDL probably to supply the growing embryo with lipids. It has been reported that especially
in the first trimester, the growing embryo depends on the supply with maternal cholesterol (67, 68). In term trophoblasts HDL degradation is decreased (61) but still higher than in other cells and tissues. This is an example showing that highly differentiated cells can degrade HDL under physiological conditions for their cholesterol supply like the CHO7 cells. Their mechanism of HDL degradation may be similar to that in CHO7 cells, since both cell lines exhibit a similar regulation pattern with the inhibitors used (compare Figs. 11 and 12). Further experiments are needed to clearly delineate the proteins involved in HDL degradation in trophoblasts.

The protein(s) mediating HDL degradation at the surface of CHO7 cells also remain to be determined. Our data show that this pathway involves loose binding of HDL to the cell membrane. After incubation with fluorescent HDL CHO7 cells exhibited a very strong cell surface staining, whereas the intracellular HDL signal did not differ substantially from that of CHOKI (Fig. 7). In contrast, cell association measured using 125I-labeled HDL did not differ significantly between CHO7 and CHOKI cells (Fig. 1). This can be explained by the different experimental procedures, since surface binding of fluorescent HDL is studied without washing the cells, whereas cell association represents the specific association of 125I-HDL after several washing steps. In these washing steps, two-thirds of the 125I-HDL associated with the cells could be removed, indicating that most HDL is loosely attached to CHO7 cells.

Interestingly, part of the protein(s) degrading HDL in CHO7 cells can be released from the cell surface by heparin, suggesting an attachment via heparan sulfate. To directly address this question of whether HSPG are involved in the HDL degradation mechanism presented here, we used genistein, which has been reported to block HSPG-mediated ligand internalization (59). Indeed, HSPG seem to play an important role in HDL degradation by CHO7 cells, since HDL degradation was severely inhibited (see Fig. 11). Lipases have been reported to play an important role in lipoprotein metabolism and are associated with the cell membrane via heparan sulfate proteoglycans. Endothelial lipase, for example, seems to play an important role in HDL and apoA-I metabolism (60, 69, 70). Overexpression of endothelial lipase in the liver leads to a significant reduction in plasma HDL cholesterol and apoA-I levels in mice (70). In our experiments, THL, an inhibitor of lipoprotein lipase activity, significantly down-regulated HDL degradation in CHO7 cells, indicating a role of lipases in this HDL degradation.

Endothelial lipase is assumed to deplete HDL particles of phospholipids through hydrolysis, which leads to the formation of smaller lipid-poor HDL. The increased catabolism of apoA-I in mice overexpressing endothelial lipase could be due to an increased dissociation of apoA-I from HDL particles when they are dismantled by endothelial lipase. Interestingly, these remodeled HDL particles show a decreased cholesteryl ester and cholesterol content (70). One can speculate that these small dense HDL could subsequently be catabolized via mechanisms like the HDL degradation pathway described here. In our experiments, however, neither triglyceride nor phospholipase activity measured in CHO7 cells after heparin release was significantly different from control values (data not shown), whereas part of the activity degrading HDL protein was released to the media (Fig. 10).

Attempts undertaken in our laboratory to isolate the genes mediating HDL degradation in CHO7 cells using cDNA expression cloning so far did not identify any candidate genes. No consistent increase in HDL degradation could be seen in CHOKI cells transfected with cDNA pools derived from CHO7 cells (data not shown).

Further studies will be necessary to elucidate the molecular details of the alternative pathway for HDL degradation discovered in CHO7 cells. This degradation process might be important for cellular cholesterol supply in certain metabolic states or in specific tissues like the placenta or may contribute to the degradation of circulating lipoprotein particles. Detailed characterization of this degradation pathway may take us a step closer to the elucidation of HDL catabolism.

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