Differential effect of combined lipase deficiency (*cld/cld*) on human hepatic lipase and lipoprotein lipase secretion

Jennifer C. Boedeker,* Mark H. Doolittle,§ and Ann L. White[1],*,†

Center for Human Nutrition* and Department of Internal Medicine,† University of Texas Southwestern Medical Center, Dallas, TX; and Lipid Research Laboratory,§ West Los Angeles VA Medical Center, Los Angeles, CA

Abstract **Combined lipase deficiency (cld) is a recessively inherited disorder in mice associated with a deficiency of LPL and hepatic lipase (HL) activity. LPL is synthesized in cld tissues but is retained in the endoplasmic reticulum (ER), whereas mouse HL (mHL) is secreted but inactive. In this study we investigated the effect of cld on the secretion of human HL (hHL) protein mass and activity. Differentiated liver cell lines were derived from cld mice and their normal heterozygous (het) littermates by transformation of hepatocytes with SV40 large T antigen. After transient transfection with lipase expression constructs, secretion of hLPL activity from cld cells was only 12% of that from het cells. In contrast, the rate of secretion of hHL activity and protein mass per unit of expressed hHL mRNA was identical for the two cell lines. An intermediate effect was observed for mHL, with a 46% reduction in secretion of activity from cld cells. The ER glucosidase inhibitor, castanospermine, decreased secretion of both hLPL and hHL from het cells by ~70%, but by only ~45% from cld cells. This is consistent with data suggesting that cld may result from a reduced concentration of the ER chaperone calnexin. In conclusion, our results demonstrate a differential effect of cld on hLPL, mHL, and hHL secretion, suggesting differential requirements for activation and exit of the enzymes from the ER.**—Boedeker, J. C., M. H. Doolittle, and A. L. White. **Differential effect of combined lipase deficiency (*cld/cld*) on human hepatic lipase and lipoprotein lipase secretion. *J. Lipid Res.* 2001.** 42: **1858–1864.**

Supplementary key words calnexin • castanospermine • endoplasmic reticulum • fetal hepatocytes • glucosidase enzymes

LPL and hepatic lipase (HL) are highly homologous enzymes (1) that play key roles in the metabolism of circulating lipoproteins. LPL is synthesized by extrahepatic tissues and hydrolyzes the triacylglycerol core of circulating VLDL and chylomicrons (2). HL is synthesized by liver parenchymal cells and mediates the conversion of VLDL remnants to LDL and of HDL subclass 2 (HDL_2) to HDL_3 (3). Both LPL and HL also directly participate in the uptake of lipoprotein particles by the liver (2, 3). Interindividual variability in the activity of LPL and HL contributes to variation in lipoprotein profiles and may influence the risk of atherosclerotic heart disease (3, 4).

Combined lipase deficiency (cld) is a recessively inherited disorder in mice that causes a severe and specific lack of LPL and HL activity (5, 6). Mice homozygous for the *cld* gene develop severe chylomicronemia on suckling and die within 72 h of birth. The *cld* locus maps to mouse chromosome 17 (5) and is unrelated to the LPL and HL structural loci (7). Cld thus provides a unique tool with which to analyze the specific factors that regulate levels of LPL and HL activity.

The absence of LPL and HL activity in cld is due to a defect in the posttranslational processing of the enzymes. Plasma LPL mass is markedly reduced in cld animals (8). LPL appears to be translated normally in adipose, heart, diaphragm muscle, and liver tissue of cld animals (9–12), but fails to attain activity. Immunofluorescence data and the structure of the N-linked carbohydrate side chains of LPL in cld cells [which remain sensitive to digestion with endoglycosidase H (endoH)] suggest retention of the enzyme in the endoplasmic reticulum (ER) (11–13). Fractionation on sucrose gradients demonstrates incorporation of LPL into high molecular weight aggregates, suggesting that LPL is misfolded in cld tissues (14, 15). One possibility is that a chaperone protein required for proper folding of newly synthesized LPL is absent or defective in cld, resulting in retention of LPL by the ER quality control processes (15). A potential candidate for an ER chaperone affected by cld is calnexin, the levels of which are reduced in microsomes from cld cells and tissues (15). Calnexin binds its substrate proteins through interaction with monoglucosylated N-linked carbohydrate side chains, which are produced on trimming of nascent N-linked sugars by ER glucosidase enzymes (16). LPL is similarly misfolded in Lec23

Abbreviations: apo, apolipoprotein; cld, combined lipase deficiency; CST, castanospermine; het, heterozygous; HL, hepatic lipase; SFM, serum-free medium.

[1] To whom correspondence should be addressed at the Department of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9046.
e-mail: Ann.White@UTSouthwestern.edu

cells, which are genetically deficient in ER glucosidase I activity and thus in calnexin interaction (15, 16). A specific defect in ER-to-Golgi transport of LPL has also been suggested to account for the lack of LPL secretion in cld (14).

The effect of cld on HL is less clear. Like LPL, HL appears to be synthesized at normal rates in cld liver and its N-linked glycans remain endoH sensitive (12). However, immunofluorescence data from Schultz, Blanchette-Mackie, and Scow (17) demonstrate secretion of HL by liver and adrenals of cld mice. The secreted HL is presumably either inactive or rapidly cleared from the circulation, as no significant HL activity was detected in the plasma of these animals (17). The question of whether HL mass is present in the plasma of cld mice has not been addressed, presumably because of the lack of anti-mouse HL (mHL) antibodies. Thus, although cld causes functional loss of both LPL and HL activity, its precise effect on the two enzymes appears to be different.

We are interested in determining the molecular mechanisms that regulate the secretion and activity of human HL (hHL). Previous results suggested an important, although nonessential, role for calnexin in hHL secretion (18). In the current study we investigated the effect of cld on the secretion of hHL protein mass and activity.

MATERIALS AND METHODS

Materials

DMEM, Williams' medium E (WME), HBSS, and FBS were from Life Technologies (Rockville, MD). Expre^{35}S^{35}S label was from DuPont NEN (Boston, MA). BSA/linoleate, insulin, and heparin were from Sigma (St. Louis, MO). Castanospermine (CST), G418, and FuGENE transfection reagent were from Roche Molecular Biochemicals (Indianapolis, IN). Rabbit anti-mouse apolipoprotein B (apoB) antibody was kindly provided by H. Hobbs (University of Texas Southwestern Medical Center, Dallas, TX). Rabbit anti-human albumin was from Dako (Carpinteria, CA), and goat anti-mouse transferrin was from Cappel (Cochranville, PA). The plasmid pSV3neo, encoding the simian virus 40 (SV40) large T antigen and neomycin resistance (19), was kindly provided by R. Lanford (Southwest Foundation for Biomedical Research, San Antonio, TX). A construct expressing the full-length hHL cDNA (20) was a generous gift from S. Santamarina-Fojo (National Heart, Lung, and Blood Institute, Bethesda, MD). A construct expressing the full-length mHL cDNA was generously provided by H. Will (Hamburg, Germany). All other reagents were of analytical grade.

Mice

Mice heterozygous for the *cld* gene were kindly provided by K. Artz (University of Texas, Austin, TX). The mice were a derivative of the original cld line (5). The non-*cld* chromosome 17 in these animals possesses a lethal mutation linked to the *cld* locus. Thus, offspring of heterozygous matings were either *cld/cld* (cld) or obligate heterozygotes (het) (K. Artz, personal communication). In addition, the *cld* locus is linked to a locus determining tail length. Pups were identified as cld or het on the basis of the absence or presence of a tail, respectively (K. Artz, personal communication).

Isolation and transformation of fetal hepatocytes

Livers were removed from 16- to 18-day fetal het and cld mice, minced, and incubated in calcium- and magnesium-free HBSS for 10 min at 37°C. The liver pieces were then transferred into calcium- and magnesium-free HBSS containing collagenase B (300 μg/ml) and incubated for 30 min at 37°C with shaking. The liver tissue was dispersed by two passages through an 18-gauge needle. The dispersed cells were pelleted at 500 *g* for 5 min and resuspended in WME containing 5% FBS. The cells were washed a further two times by centrifugation in the same medium and then plated into 35-mm dishes. Three hours after plating the cells were washed with PBS and transferred into a defined serum-free medium [SFM (21)] that we previously used to culture adult mouse hepatocytes (22). Although initially sparse, the isolated cells rapidly proliferated and were confluent 24–48 h after isolation. This is in contrast to adult mouse hepatocytes, which do not proliferate in culture (A. L. White, unpublished observation). The ability of the cells to divide was limited, however, as after a single passage the cells stopped dividing. The cells remained viable in the SFM for at least 30 days (longest time point analyzed; data not shown).

To derive permanent liver cell lines, fetal hepatocytes were transfected 24 h after isolation with pSV3neo as previously described (23). Two days after transfection, cells were placed in G418 (400 μg/ml) to kill untransfected cells and allow proliferation of transformed colonies. The cell lines used in this study were derived from multiple individual colonies. Once cell lines were established, the transformed cells were adapted to culture in DMEM-10% FBS. The cell lines grew rapidly and were passaged at a 1:40 ratio two or three times weekly.

To confirm the genotypes of the derived cell lines, DNA was prepared from cld and het cells, using a QIAquick blood kit (Qiagen, Chatsworth, CA). PCR was performed with ^{32}P-labeled PCR primers D17mit63 and D17mit83 (Research Genetics, Huntsville, AL), to markers on mouse chromosome 17, using standard techniques. Products were resolved on a 7% acrylamide-30% formamide gel.

Metabolic labeling and immunoprecipitation

Cultures were labeled overnight in medium [SFM or DMEM containing BSA/linoleate (0.5 mg/ml, 0.79% linoleic acid) and insulin (5 μg/ml)] containing one-tenth the normal concentration of methionine and cysteine and Expre^{35}S^{35}S label (125 μCi/ml). Medium and cell lysates were collected and immunoprecipitated as described previously (18). Bands on autoradiographs were quantified by densitometric scanning as previously described (18).

Transfection

Cld and het liver cell lines were transfected with plasmids encoding hHL (20) or hLPL (15), using FuGENE transfection reagent according to the manufacturer instructions. For 35- and 60-mm dishes of cells, 2 and 6 μg of plasmid DNA were used, respectively. In some experiments, cotransfection with a plasmid (pCH110; Roche Molecular Biochemicals) encoding the β-galactosidase gene was used to control for transfection efficiency. A 10:1 ratio of test plasmid:pCH110 was used. β-Galactosidase activity in cell lysates was determined with a commercial kit according to the manufacturer instructions (Roche Molecular Biochemicals).

Lipase activity assays

HL and LPL activities were measured in medium containing heparin (10 U/ml) conditioned by overnight incubation with transfected cells. The assays were performed as previously described (18, 24), using as substrate emulsions of radiolabeled triolein prepared by sonication. Samples were assayed in duplicate and values obtained were normalized either to total cell protein determined by bicinchoninic acid assay (Pierce, Rockford, IL)

or to the relative mass of HL in the culture medium determined by immunoprecipitation of medium labeled overnight as described above. Results were calculated as milliunits of activity (nanomoles of free fatty acid hydrolyzed per minute) per milligram of cell protein, or per unit of HL mass.

Quantitation of hHL mRNA

Total RNA was prepared from transfected cells 40 h after transfection, using TriZol (GIBCO, Grand Island, NY) according to the manufacturer instructions. HL mRNA was then quantified by ribonuclease protection assay (RPA). Radiolabeled antisense RNA probes against hHL and endogenous mouse actin mRNA were prepared and gel purified with a MAXIscript in vitro transcription kit (Ambion, Austin, TX). The 380-base antisense HL probe was made by T3 polymerase-directed transcription, using as template a *Bam*HI-linearized Bluescript plasmid containing a 959-bp fragment of the hHL cDNA (hHL-SK), kindly provided by J. Cohen (University of Texas Southwestern Medical Center). The 245-base β-actin probe was prepared with the pTRI-β-actin mouse plasmid (Ambion) and T3 polymerase.

RPA analysis was performed with an RPA III kit (Ambion). Aliquots of total RNA were digested with RNase-free DNase I (Roche Molecular Biochemicals) for 30 min at 37°C to remove any contaminating plasmid DNA. The RNA was then hybridized to the radiolabeled probes overnight at 56°C and digested with RNase A/T1, according to kit instructions. Protected fragments were analyzed on 5% acrylamide-urea gels. In each assay, yeast RNA and undigested probe controls were included. In addition, to control for the possibility of remaining plasmid contamination, RNA prepared from cells transfected with hHL-SK was included as this plasmid would give a positive signal in the RPA, but would not give rise to mRNA. HL mRNA levels were quantified by densitometry (18), and normalized to β-actin mRNA levels.

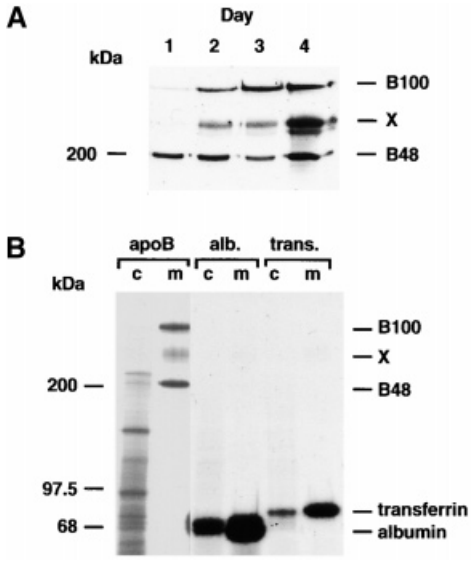

Fig. 1. Characterization of fetal mouse hepatocytes. Hepatocytes from cld and het fetal mice were isolated and cultured as described in Materials and Methods. A: Aliquots of medium collected every 24 h between 1 and 4 days of culture were analyzed for the presence of mouse apoB by immunoblotting, as described (25). The positions of apoB-100, apoB-48, the 200-kDa marker, and a nonspecifically recognized protein (X) are indicated. B: Primary hepatocytes were labeled overnight with [^{35}S]methionine, and apoB, albumin, and transferrin were immunoprecipitated from the cell lysates (c) and culture medium (m), as described in Materials and Methods, and analyzed by 4–10% SDS-PAGE. The positions of molecular weight standards and immunoprecipitated proteins are indicated. X denotes a protein nonspecifically immunoprecipitated from the culture medium. A longer exposure of the autoradiograph is shown for apoB than for albumin and transferrin.

RESULTS

Production of stable het and cld liver cell lines

To analyze the effect of cld on hepatic secretion of hHL, we derived transformed liver cell lines from cld mice and their normal (het) littermates. Primary hepatocytes were isolated from 16- to 18-day fetal cld and het mice and were cultured in a defined SFM demonstrated to maintain adult mouse hepatocytes in a highly differentiated state (22) (for details, see Materials and Methods).

To confirm that the isolated cells were hepatocytes, we analyzed their ability to secrete liver-specific proteins. ApoB represents a highly differentiated marker for hepatocytes. Immunoblotting of culture medium demonstrated the expression of both apoB-100 and apoB-48 for at least 4 days in culture (the longest time point analyzed; **Fig. 1A**) (25). Immunoprecipitation of medium from cultures labeled for 16 h with [^{35}S]methionine confirmed the expression of apoB-100 and apoB-48, and also demonstrated secretion of the liver-specific proteins albumin and transferrin (Fig. 1B).

Stable cell lines were established from the het and cld hepatocytes by transfection with a plasmid encoding the SV40 large T antigen (see Materials and Methods for details). The genotypes of the derived cld and het cell lines were confirmed by PCR (**Fig. 2A**). To investigate the differentiated status of the transformed cell lines, the presence of apoB, albumin, and transferrin in radiolabeled culture medium was examined by immunoprecipitation (Fig. 2B). Secretion of apoB from the cells was no longer detectable (Fig. 2B). We have commonly observed loss of apoB secretion on transformation of mouse hepatocytes (J. C. Boedeker and A. L. White, unpublished observation). However, both albumin and transferrin were readily detected in the media of the cld and het cell lines (Fig. 2B).

For both the het and cld primary hepatocytes and the het and cld cell lines, HL and LPL activity secreted in the presence of heparin over a 24-h period was not significantly above the background of the lipase assays (data not shown). This suggests either that expression of the endogenous LPL and HL genes was not maintained after isolation and culture of the hepatocytes, or that expression of the genes had not yet been activated in the fetal hepatocytes.

Cld inhibits secretion of hLPL activity from liver cells

To confirm the cld and het phenotypes of our cell lines, we examined the secretion of LPL activity from cells transiently transfected with an hLPL expression construct (**Fig. 3A**). Medium from untransfected cells was used as a background control. Cotransfection with a β-galactosidase expression plasmid was used to control for transfection ef-

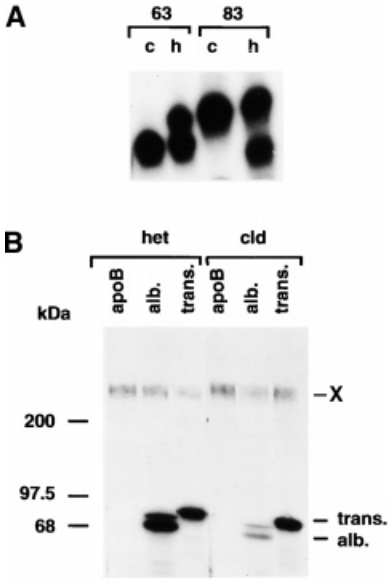

Fig. 2. Characterization of transformed het and cld liver cell lines. Transformed cell lines were derived from fetal hepatocyte cultures as described in Materials and Methods. A: The genotypes of the cld (c) and het (h) cell lines were confirmed by PCR, using ^{32}P-labeled primers D17mit63 (63) and D17mit83 (83) as described in Materials and Methods. B: Transformed het and cld cells were labeled overnight and apoB, albumin (alb.), and transferrin (trans.) were immunoprecipitated from the culture medium as described in the legend to Fig. 1. X denotes a protein nonspecifically immunoprecipitated from the culture medium.

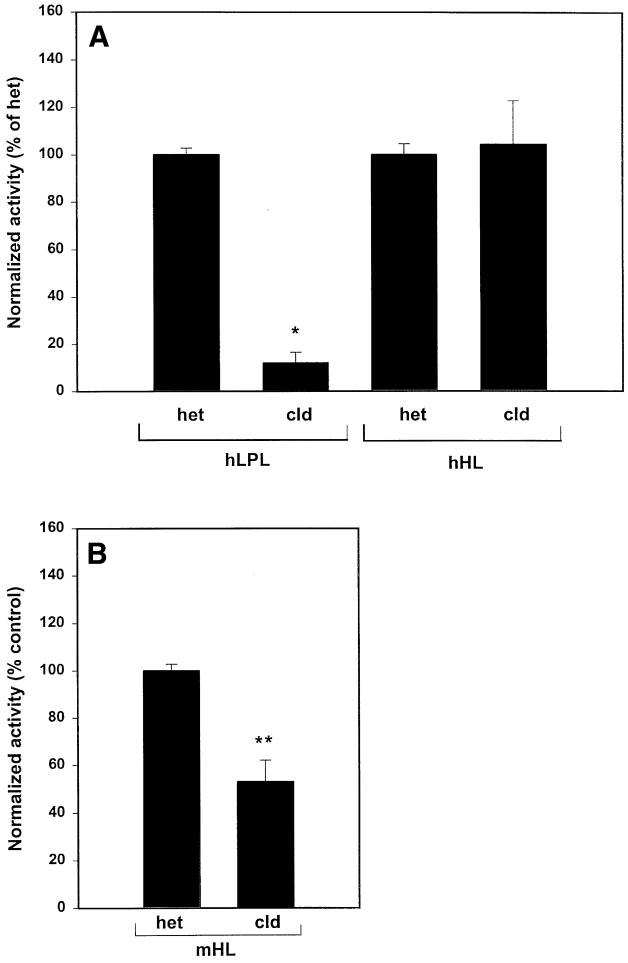

Fig. 3. Cld differentially affects secretion of hLPL and human and mouse HL. A: Het and cld liver cell lines were transiently cotransfected with constructs expressing hLPL or hHL and a β-galactosidase expression construct. Secreted LPL and HL activity was assayed and normalized to cellular β-galactosidase activity, as described in Materials and Methods. Values (means ± SE of two or three independent experiments performed in duplicate) are expressed relative to values in het cells (taken as 100%). B: Experiments using a mHL cDNA expression construct were performed exactly as described in (A). Values represent means ± SE of two independent experiments performed in quadruplicate. * $P = 1 \times 10^{-8}$; ** $P = 5 \times 10^{-5}$.

ficiency. Secretion of LPL activity from cld cells was only 12 ± 11% of that secreted from het cells (Fig. 3A; $P = 1 \times 10^{-8}$) when normalized to β-galactosidase activity in the cell lysates. These results are consistent with previous observations (15), and confirm the cld and het phenotypes of our liver cell lines.

hHL is secreted normally from cld cells

To examine the effect of cld on secretion of hHL activity, we performed transient transfection experiments using β-galactosidase as a control, as described above for LPL. Surprisingly, we found that secretion of hHL activity from the cld cells was similar (104 ± 36%; $P = 0.82$) to that secreted from het cells when normalized to β-galactosidase activity (Fig. 3A). These results suggest that hHL is secreted normally from cld cells, and demonstrate a differential effect of cld on hLPL and hHL secretion.

To further examine the secretion of hHL from cld cells, we performed a careful analysis of hHL mRNA levels and secretion of both hHL protein mass and activity in cld and het cell cultures transiently transfected with hHL (**Fig. 4**).

hHL mRNA levels were quantified by ribonuclease protection using a probe from the 5′ end of hHL, and normalized using a probe against endogenous mouse actin mRNA. Results of a representative RPA are shown in Fig. 4A. The levels of hHL mRNA achieved in the het and cld cell lines were almost identical (Fig. 4A). We then compared the levels of hHL mRNA with the amount of hHL activity secreted into the medium of the transfected cells. Over three independent transfection experiments, the amount of secreted hHL activity per unit of hHL mRNA mass for the cld cells was 108 ± 15% (mean ± SD) of that for het cells ($P = 0.396$).

Data from Schultz, Blanchette-Mackie, and Scow (17) suggest a differential effect of cld on the secretion of mHL protein mass and activity. To examine the effect of cld on the specific activity of the secreted hHL, transiently transfected het and cld liver cells were radiolabeled with [^{35}S]methionine and used to determine secretion of hHL mass by immunoprecipitation and SDS-PAGE. hHL protein mass was readily detected in the medium of both transfected cell types (Fig. 4B). In the experiment shown, 1.48-fold more hHL protein mass was secreted from het versus cld cells. This correlated with a 1.58-fold higher

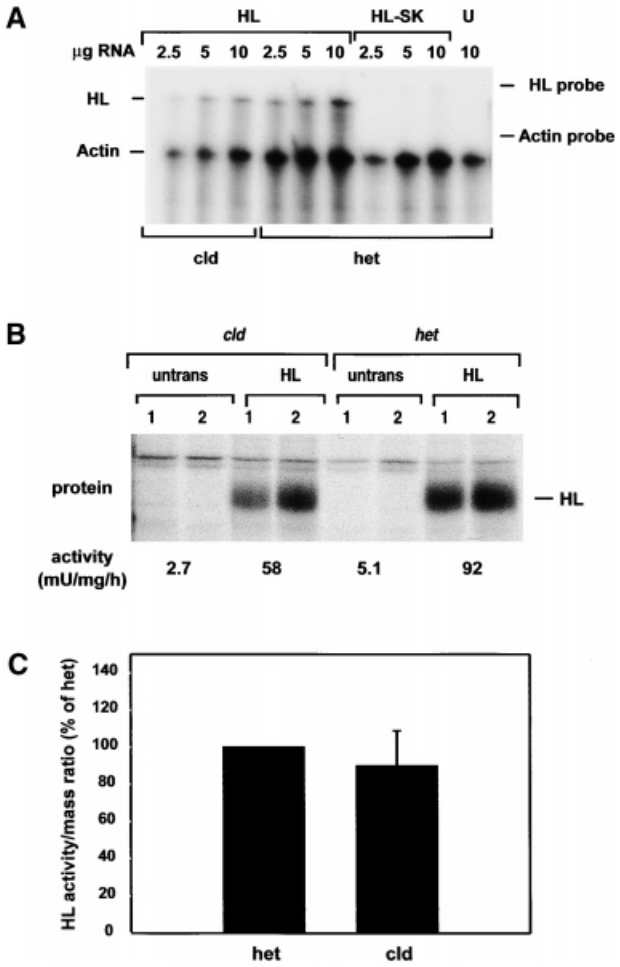

Fig. 4. hHL is secreted normally from cld cells. A: hHL mRNA levels were analyzed in untransfected cells (U) or in het and cld cells transiently transfected with wild-type HL or the HL-SK construct, by RPA using endogenous mouse actin mRNA as an internal control (see Materials and Methods). The positions of the HL and actin probes and protected fragments are indicated. The autoradiograph shown is representative of three independent experiments. B: Cld and het liver cell lines were transiently transfected with hHL and then split into smaller dishes. Duplicate plates of untransfected (untrans) and hHL-transfected (HL) cells were labeled overnight with [^{35}S]methionine and hHL secreted into the culture medium was immunoprecipitated as described in Materials and Methods. Samples were analyzed by 8% SDS-PAGE. The position of hHL is indicated. The remaining plate of each cell type was incubated in heparin overnight and hHL activity secreted into the culture medium was assayed in duplicate as described in Materials and Methods. Activity is presented as milliunits per milligram per hour. C: The specific activity of hHL secreted from cld and het cells was calculated as described in Materials and Methods. Results for cld cell cultures are presented as a percentage of values obtained for het cell cultures. The error bar represents the SE for five independent experiments.

secretion of hHL activity (Fig. 4B), suggesting that hHL secreted from cld and het cells had similar specific activity. Over five independent experiments, the specific activity of hHL secreted from cld cells was 90 ± 19% (mean ± SE) of that secreted from het cells ($P = 0.61$; Fig. 4C). Thus, hHL appears to be secreted at normal rates from cld cells and with normal specific activity.

Cld has an intermediate effect on the secretion of mHL activity

Data from Schultz, Blanchette-Mackie, and Scow (17) suggest that mHL is secreted from cld cells but is inactive. Because our data demonstrate secretion of normally active hHL from our cld cell line, this suggests a differential effect of cld on the mouse and human HL enzymes. To directly compare the effect of cld on secretion of hHL and mHL, we examined the secretion of mHL from our het and cld cell lines transiently transfected with a mouse cDNA expression construct. When normalized to β-galactosidase coexpression levels, secretion of mHL activity from the cld cells was 54 ± 20% (mean ± SD) of that secreted from het cells ($P = 5 \times 10^{-5}$; Fig. 3B). Human LPL (hLPL)-transfected cld cells analyzed in parallel secreted 17 ± 9% of lipase activity secreted by LPL-transfected het cells ($P = 1 \times 10^{-5}$; data not shown). The effect of cld on hLPL was significantly larger than its effect on mHL ($P < 0.01$). In contrast, a small increase in hHL secretion from cld cells was observed compared with het cells in these experiments (data not shown). Our results therefore suggest that cld has an intermediate effect on the secretion of mHL activity compared with hLPL and hHL activity. We were unable to analyze the secretion of mHL mass in these experiments because of the lack of a suitable antibody.

Inhibition of ER glucosidase activity decreases hLPL and hHL secretion from both het and cld cells

Studies by Briquet-Laugier et al. (15) suggest that cld may result from a functional defect in the ER chaperone calnexin; microsomes isolated from cld cells have a reduced concentration of calnexin, and hLPL is similarly misfolded in cld cells and the ER glucosidase I-deficient cell line Lec23 (15). Processing by ER glucosidase enzymes is necessary for interaction of most glycoproteins with calnexin (16).

To investigate the role of calnexin in cld, we examined the effect of the ER glucosidase inhibitor castanospermine (CST) on hHL and hLPL secretion from het and cld cells (**Fig. 5**). In het cells, CST caused a virtually identical reduction in hHL and hLPL secretion to 30 ± 22% and 28 ± 7% of control values, respectively, suggesting an identical requirement for ER glucosidase processing for secretion. In cld cells, the effect of CST was also similar for hHL and hLPL, but was significantly less than in het cells; hHL and hLPL secretion were reduced to 56 ± 23% and 57 ± 17% of control values, respectively (Fig. 5). These results are consistent with an effect of cld on calnexin function, but suggest that other glucosidase-dependent processes may also be important in lipase secretion.

DISCUSSION

In this study we examined the effect of cld on the secretion of hHL mass and activity, using liver cell lines derived from cld mice and their normal (het) littermates. We found that hHL was secreted with similar efficiency and with similar specific activity from cld and het cells. In con-

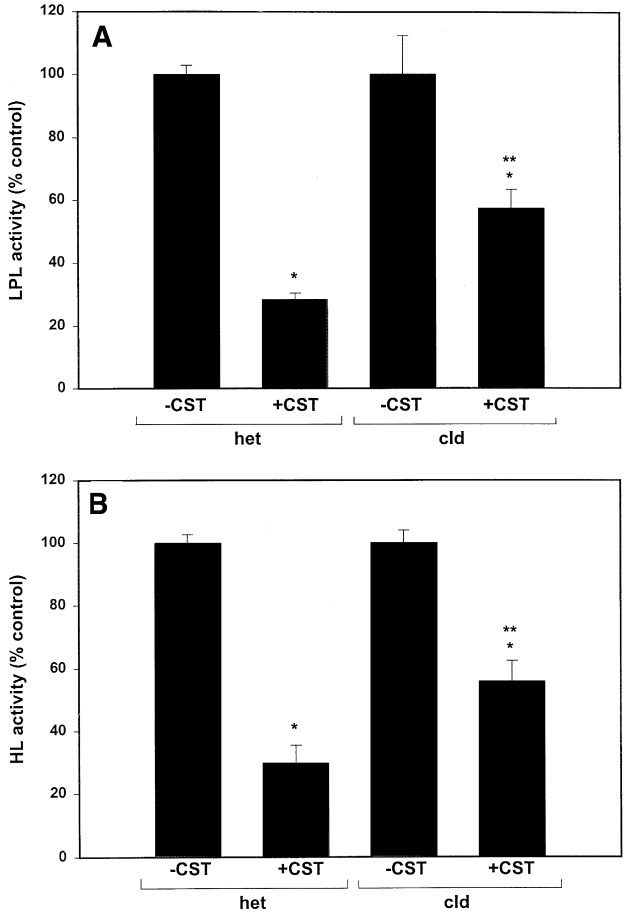

Fig. 5. Effect of ER glucosidase inhibition on hHL and hLPL secretion from het and cld cells. het and cld cells were transiently transfected with hLPL (A) or hHL (B) and then split into smaller dishes. Forty-eight hours after transfection, cells were placed in fresh medium containing heparin in the presence or absence of 1 mM castanospermine (CST) and the incubation was continued for 5 h. LPL and HL activity secreted into the medium was then assayed as described in Materials and Methods. Results are expressed as a percentage of activity secreted in the absence of CST and represent the means ± SE of at least three independent experiments performed in duplicate. * $P < 0.01$ versus untreated cells; ** $P < 0.01$ versus het plus CST.

trast, secretion of hLPL activity from cld cells was reduced by 88% compared with het cells. An intermediate effect on the secretion of mHL activity was observed, with activity secreted from cld cells reduced by 46%. Our studies demonstrate that cld differentially affects hLPL, hHL, and mHL secretion, suggesting differential requirements for activation and exit of these enzymes from the ER.

Our results demonstrating apparently normal secretion of hHL from cld liver cells are in contrast to those obtained for LPL and mHL. hLPL is retained in the ER of cld cells and appears to be misfolded, suggesting that a factor required for correct folding of hLPL is defective or absent in cld cells (15). Studies by Shultz, Blanchette-Mackie, and Scow (17) suggest that at least a portion of mHL is secreted from cld liver and adrenals, but is presumably either inactive or rapidly cleared from the circulation, as no HL activity is detected in the plasma of cld animals (17). Results in the present study also demonstrate secretion of mHL from cld cells, and, moreover, suggest that the secreted enzyme is active. A 46% reduction in the secretion of mHL activity from cld cells was observed compared with het cells, however, although this was significantly less than the >80% reduction observed in LPL secretion. Because of the lack of a suitable antibody we were unable to determine whether the reduction in mHL secretion was due to a decrease in secretion of mHL protein mass or to a decrease in the specific activity of the secreted enzyme. The results do suggest, however, that although some mHL may fold sufficiently well to escape the ER quality control process in cld cells, the putative "folding factor" that is defective in cld is presumably required for normal function of the enzyme in vivo. This putative factor thus appears to be required to different extents for hLPL, mHL, and hHL secretion and activity, suggesting differential requirements for activation and exit of the different lipases from the ER.

hLPL and mouse and human HL are part of a superfamily of lipase enzymes that share similar structures and functions (1). Each enzyme has a large N-terminal domain that contains the catalytic triad covered by a hinged loop ("lipase lid"), and a smaller C-terminal domain that is required for substrate interaction and determines heparin affinity. Disulfide-bridging cysteines are conserved between the three enzymes, as are two N-linked glycosylation sites, one in the N-terminal region (Asn-56 in hHL, Asn-57 in mHL, and Asn-43 in hLPL), and one in the C-terminal region (Asn-375 in hHL, Asn-376 in mHL, and Asn-359 in hLPL) (1, 9, 26). Despite their overall similar structures there are many amino acid differences between LPL and HL that could potentially account for the differential effect of cld on the enzymes (1). The human and mouse HL enzymes, on the other hand, are much more closely related (86% amino acid homology vs. 42% between mLPL and mHL) (9, 26). The most striking difference in amino acid sequence between human and mouse HL occurs at the carboxyl-terminal end of the proteins with the addition of 10 amino acids to the mouse enzyme (9, 26). The C-terminal region of hHL contains a postulated heparin-binding domain, and differences in this region may contribute to the markedly reduced heparin binding exhibited by mHL (9). It seems unlikely that the difference in heparin binding accounts for the differential effect of cld on hHL and mHL as hLPL binds heparin more avidly than either mHL or hHL and is more dramatically affected by cld than either HL enzyme. It is possible, however, that the additional amino acids at the C-terminal end of mHL make it more susceptible than hHL to misfolding in cld cells.

One striking difference between hHL and hLPL and mHL is the presence of two additional N-linked glycosylation sites in hHL at positions 20 and 340 of the mature protein (1). N-linked glycans can help nascent proteins to fold in the ER by increasing overall hydrophilicity and by mediating interaction with the lectin ER chaperones calnexin, calreticulin, and ERp57 (16, 27, 28). It is possible

that the additional carbohydrate on hHL may enhance folding of the enzyme and make it less susceptible than hLPL and mHL to misfolding in cld cells. Previous studies suggested an important, although nonessential, role for calnexin in hHL secretion (18). Calnexin has also been suggested as a potential candidate for the folding factor that is defective in cld as levels of the chaperone in microsomes isolated from cld tissues and cells are reduced (15). The ER glucosidase inhibitor CST prevents interaction of nascent proteins with calnexin, calreticulin, and ERp57 (16, 27, 28). In the current study we found that CST had a significantly less inhibitory effect on hLPL and hHL secretion from cld cells (~45% for both enzymes) than from het cells (~70% for both enzymes), which is consistent with a decreased calnexin function in cld cells. If calnexin function is indeed compromised in cld, the ability of CST to decrease hHL and further decrease hLPL secretion from cld cells would, however, indicate that other glucosidase-dependent processes (such as interaction with calreticulin or ERp57) play an important role in lipase secretion. Future studies involving addition and mutagenesis of existing N-linked glycosylation sites and domain swapping between lipase enzymes will be designed to explore the specific structural features responsible for the differential effect of cld on hLPL, hHL, and mHL secretion.

In conclusion, our studies demonstrate that, in contrast to hLPL and mHL, cld cells are capable of secreting hHL with normal specific activity and at normal rates. These data provide further support for the differential requirements for posttranslational processing among the lipases and provide additional insight into the defect responsible for cld.

This work was supported by the Southwestern Medical Foundation and the Moss Heart Foundation (Dallas, TX). We are grateful to Helen Hobbs for critical reading of the manuscript.

Manuscript received 8 March 2001 and in revised form 27 June 2001.

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