Human liposarcoma cell line, SW872, secretes cholesteryl ester transfer protein in response to cholesterol

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Summary Cholesteryl ester transfer protein (CETP) mediates the exchange of phospholipids and neutral lipids between the plasma lipoproteins, and plays an important role in high density lipoprotein (HDL) metabolism. While there are reports of low-level CETP secretion from cultured cells, the lack of a good model cell line has hampered the detailed study of CETP regulation and secretion. In this study, we have found that the human liposarcoma cell line, SW872, secretes cholesteryl ester transfer protein at levels substantially higher than observed from other cell lines. The secretion of CETP from this adipose-derived cell was up-regulated by 25-OH cholesterol and by low density lipoprotein (LDL) cholesterol in a concentration-dependent manner. Analysis of both full length and exon 9-deleted CETP mRNA demonstrated increases in response to LDL and 25-OH cholesterol, providing evidence for regulation at the message level. Our results suggest that the CETP-producing SW872 cell line may provide a model in which to study the regulation of this important modulator of lipoprotein metabolism.—**Richardson, M. A., D. T. Berg, P. A. Johnston, D. McClure, and B. W. Grinnell.** Human liposarcoma cell line, SW872, secretes cholesteryl ester transfer protein in response to cholesterol. *J. Lipid Res.* 1996. **37:** 1162–1166.

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Cholesteryl ester transfer protein (CETP) is a 74 kDa hydrophobic glycoprotein that mediates the exchange of phospholipids and neutral lipids between the plasma lipoproteins (reviewed in refs. 1–3). CETP plays a critical role in high density lipoprotein (HDL) metabolism though the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins, low density lipoprotein (LDL), and very low density lipoprotein (VLDL), with a reciprocal transfer of triacylglycerol. From the study of CETP-deficient families (reviewed in ref. 4), and through the inhibition of CETP in animal models (5, 6), there is a clear association between a reduction in CETP and an increase in HDL. Moreover, the over-expression of CETP in transgenic mice resulted in a decrease in HDL (7, 8) and increased susceptibility to the development of atherosclerotic lesions (8). The reciprocal relationship between CETP activity and HDL levels may be exploited in therapeutic strategies designed to increase the HDL cholesterol.

CETP mRNA levels have been shown to increase in response to dietary cholesterol in several species (reviewed in ref. 1), and a mini-gene for human CETP has been studied in transgenic animals and was shown to be cholesterol regulated (9). However, there have been few detailed studies on the genetic regulation of CETP, in part because of the lack of cell lines demonstrated to regulate and secrete easily measurable levels of CETP in vitro. There are several reports that the human HepG2 cell line secretes CETP (10, 11) and that low level CETP mRNA can be detected by PCR (12). In addition, vectorial secretion of CETP activity has been reported in the human epithelial CaCo-2 cell line. However, the reported level of expression in each of these cases was very low. In fact, studies have shown that primary hepatic cell cultures rapidly lose the ability to express CETP apparently through the loss of the transcription factor C/EBP (13).

The most abundant source of CETP mRNA in humans is the liver, spleen, and adipose tissue (1). Adipose tissues appear to show the most conserved expression in different species (14) and, while hepatic synthesis is felt to be a major source of plasma CETP in primates, it has been suggested that adipose tissue synthesis may also contribute to plasma levels (1). In the present studies, we sought to identify a cell line capable of secreting easily detectable levels of CETP. Although a variety of cells, including a survey of human hepatoma lines and virally transformed human liver lines showed extremely low levels of CETP, we identified an adipocyte-derived human liposarcoma cell line SW872 that produced CETP at levels 50-times those of the HepG2 cell line. We demonstrate that CETP expression from this line could be repressed by serum-starvation and subsequently induced by LDL in a concentration-dependent manner. Further, we show that the increase in CETP activity in the culture medium correlates with a concomitant increase in mRNA level. Our data suggest that the SW872 cell line could be a useful tool for better understanding the regulation and physiological control of human CETP.

Abbreviations: CETP, cholesteryl ester transfer protein; LDL, low density lipoprotein; HDL, high density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; RPA, ribonuclease protection assay; GAPDH, glyceraldehyde phosphate dehydrogenase; ΔE9, exon 9-deleted.

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MATERIALS AND METHODS

Materials

The human hepatocellular carcinoma lines, HepG2, HepG3B, and SK-Hep-1, the human colon adenocarcinoma, CaCo2, and the human liposarcoma cell line, SW872, were purchased from American Type Culture Collection (Rockville, MD). A transformed primary human embryonic liver line was constructed using an origin-defective SV40 plasmid as described previously (15). The BCA Protein Assay Reagent was purchased from Pierce (Rockford, IL), and the CETP Diagnescent Kit was from Diagnescent Technologies (Yonkers, NY). The human low density lipoprotein was obtained from PerImmune, Inc. (Rockville, MD). Dulbecco's modified Eagle's/Ham's F12 medium (DMEM/F-12) and gentamicin (600-5750AD) were from GIBCO BRL; HEPES (16926) was from United States Biochemical; fetal bovine serum (A-1111-L) was from HyClone; cell culture medium DMEM/F-12(3:1) and fetal bovine serum were from Life Technologies (Gaithersburg, MD). Pentex FAF bovine serum albumin (82-002) and Pentex human transferrin (82-316) were from Miles Scientific, and bovine insulin was from Eli Lilly & Company. 25-OH-cholesterol was from Sigma. All other reagents were of the highest quality available.

Cell culture and CETP assay

Cells were cultured in DMEM/F-12 (3:1), 5% fetal bovine serum, 20 mM HEPES, and 50 μg/ml gentamicin, 5% CO_2 at 37°C. For assay of secreted CETP activity, cells were seeded into six-well tissue culture plates (5×10^5 cells/well) or 96-well plates (10^3 cells/well) and incubated 16–24 h at 37°C, 5% CO_2. After the monolayers achieved 70–80% confluence, the spent medium was aspirated from the wells and serum-free medium was added (DMEM/F12 (3:1), 100 μg/ml fatty acid-free BSA/oleic acid, 20 mM HEPES, pH 7.5, 50 μg/ml gentamicin, 1 μg/ml human transferrin, and 1 μg/ml bovine insulin). After incubation for 16–24 h, the medium was removed and assayed for CETP activity. In some experiments, the cells were serum-starved by being maintained in the serum-free medium for 8–24 h prior to treatment. The effect of human LDL and 25-OH cholesterol was determined by the addition of varying levels of each in serum-free medium 16–24 h prior to the determination of CETP activity in the culture medium. The level of CETP activity was determined from 100 μl of conditioned media by adding 100 μl of the CETP Diagnescent assay cocktail (20 mM Tris, pH 7.4, 300 mM NaCl, 4 mM EDTA, 4 μl NBD-cholesteryl linoleate, and 4 μl human VLDL), incubating at 37°C for 3 h, and determining fluorescence transfer using either a CytoFluor 2350 or CytoFluor II, Fluorescence Measurement System (PerSeptive Biosystems, Framingham, Massachusetts). With cells cultured in a 96-well plate, CETP activity in the medium could be assayed directly in the same plate without removal of cells, with no change in results. For each culture condition, control wells without cells were assayed to control for possible effects of different media on transfer activity. The level of CETP produced was expressed as the fluorescent units/μg of cell protein, with protein levels determined by solubilizing washed cell monolayers in 0.2 N NaOH, 0.5% SDS, and assaying with Pierce BCA Protein Assay Reagent.

Ribonuclease protection assay

Total RNA was isolated as described by Chomczynski and Sacchi (16) using the Biotecx Ultraspec RNA isolation system. CETP mRNA levels were analyzed by a ribonuclease protection assay using the Ambion RPA II Kit and an antisense riboprobe synthesized to the 3′ end of CETP. To control for the amount of RNA used in the ribonuclease protection assays (RPA), an antisense riboprobe synthesized to the 3′ end of glyceraldehyde phosphate dehydrogenase (GAPDH) was used. The 3′ antisense CETP riboprobe was prepared from the synthetic CETP structural gene (which was supplied by A. Tall) (9). NFR-CETP was cloned into pBluescriptII KST at the SpeI to SalI sites. The human CETP cDNA was excised on a 751 bp EcoRV fragment and was cloned into the Sma I site of site of pGEM3Z (Promega) to make pGEM3Z-CETP20. pGEM3Z-CETP20 was purified, di-

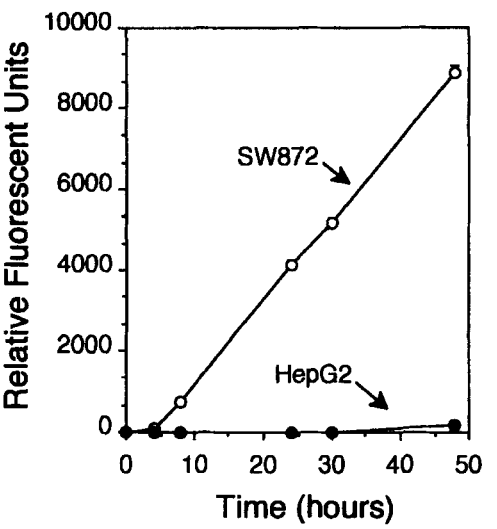

Fig. 1. Time course of CETP activity secretion in SW872 and HepG2 cells. SW872 and HepG2 cells were grown in serum-containing medium to confluence. The cell were washed, serum-free medium was added, and samples were removed at various times for CETP activity determinations as described under the Materials and Methods. The CETP activity is reported in relative fluorescent units/μg cellular protein.

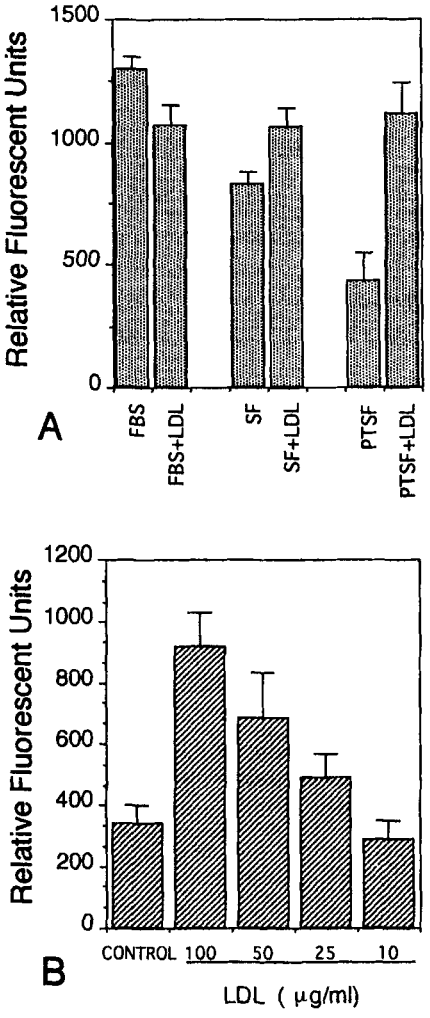

Fig. 2. Secreted CETP activity is regulated by LDL in SW872 cells. A) Confluent cultures of SW872 cells were treated with 100 µg/ml LDL in the presence of fetal bovine serum (FBS), in serum-free medium (SF), or in serum-free medium after an 8-h serum-starvation (PTSF). After 16 h at 37°C, the levels of CETP secreted into the medium were determined on 100-µl samples of conditioned medium. B) Confluent cultures of SW872 cells were pretreated with serum-free medium for 8 h, the medium was replaced with fresh serum-free medium containing varying levels of LDL cholesterol (0, 10, 25, 50, and 100 µg/ml LDL), and 16 h later 100-µl samples were assayed for CETP activity. The level of CETP activity is expressed in relative fluorescent units and the results are the mean ± SEM of three determinations.

gested with Sty I, and the 3226 base pair fragment comprised of the SP6 RNA polymerase promoter positioned to synthesize runoff transcripts of the 3′ end of CETP was isolated and purified using the Bio-Rad Prep-a-gene DNA Purification Kit. The 3′ antisense CETP riboprobe was transcribed with SP6 RNA polymerase and [α-^{32}P]CTP using the Promega Riboprobe Gemini II kit to produce a 323 nucleotide transcript that would protect a 277 base fragment corresponding to the wild type CETP mRNA and a 217 bp fragment corresponding to the ΔE9 CETP mRNA. An antisense riboprobe to the 3′ end of GAPDH was similarly prepared to use as a control for the amount of RNA in the RPA. The cDNA for GAPDH was obtained from the ATCC cloned in pHcGAP (17). A 548 base pair Xba I to Hind III fragment of the 3′ end of GAPDH was cloned into the Xba I to Hind III site of the pGEM4Z (Promega) polylinker to make pGEM4Z-GAPDH. PGEM4Z-GAPDH was linearized with Hind III and transcribed with SP6 RNA polymerase and [α-^{32}P]CTP using the Promega Riboprobe Gemini II kit to produce a 580 nucleotide transcript that would protect a 543 base pair fragment. The protected fragments of CETP and GAPDH were resolved by 5% TBE-urea polyacrylamide gel electrophoresis MiniPlus SepraGels (Integrated Separation Systems).

RESULTS AND DISCUSSION

Human liposarcoma SW872 cells secrete CETP

We examined a number of cell lines originating from different human tissues in search of a cell line that secreted appreciable levels of CETP activity. Most notably, we investigated the human hepatocellular carcinoma lines, HepG2, HepG3B, and SK-Hep-1, the human colon adenocarcinoma CaCo2, the human embryonic kidney line 293, and SV40-transformed human embryonic liver cells. While trace levels of activity were detected in HepG2 and CaCo2 cells, none of the these cell lines secreted easily detectable and reproducible levels of CETP activity as judged by the fluorescent transfer assay. With regard to the commonly used HepG2 line, we examined five different isolates including a stock of the original Wistar isolate, the ATCC #HB8065 culture, and three additional lab strains of varying histories. In addition, we subcloned the ATCC stock and examined multiple clonal isolates. None of the lines or subclones secreted significant or reproducible levels of CETP activity.

Because adipose tissue is a major source of CETP mRNA in some animal species, and is the tissue that probably shows the most conserved expression (2), we examined the human liposarcoma cell line, SW872, and found that it produced significant and reproducible levels of CETP activity. As shown in **Fig. 1,** the amount of CETP activity secreted into the medium increased with time in culture. For comparison, we examined the kinetics of CETP secretion from HepG2 cells under identical culture conditions and cell densities. While significant CETP activity could be detected above background from the HepG2 cells at the 48 h time point, the levels were ~50-times less than those observed with the SW872 cell line. The SW872 cell line produced relatively

high levels of CETP, ranging from 20 to 80 ng/10^6 cells per 24 h in serum-free medium.

Low density lipoprotein increases secreted CETP activity

Because dietary cholesterol has been shown to increase CETP in several species, experiments were performed to determine whether CETP expression in SW872 liposarcoma cells responded to cholesterol. In initial experiments (**Fig. 2A**), we examined the effect of LDL [100 μg/ml] on CETP secretion from cells over a 24-h period in the presence of fetal bovine serum (FBS), in serum-free medium (SF) and in SF medium after serum-starvation for 8 h (PTSF). As shown, there was a significant decrease in CETP produced in SF medium and an even greater decrease under serum-starved conditions. LDL treatment resulted in a 2.7-fold increase in CETP activity in the culture medium in the serum-starved cells. However, in the presence of serum, there was no increase in CETP by LDL treatment. These data suggest that in serum, CETP levels are already maximally induced and thus not responsive to additional cholesterol. Further, serum-starvation places the cells in an uninduced state that is responsive to added cholesterol. As shown in Fig. 2B, the effect of LDL on CETP induction was concentration-dependent, with 100 μg/ml consistently inducing levels by 2.5- to 3-fold. CETP also be could induced to a similar degree by 25-OH cholesterol (2–10 μg/ml), although the fold induction was more variable than observed with LDL (data not shown).

25-OH cholesterol increases CETP mRNA

To determine whether the observed effects of cholesterol on CETP expression from the SW872 cells were due to changes in mRNA levels, cells were treated with LDL or 25-OH cholesterol and CETP mRNA was detected using a ribonuclease protection assay (RPA). The probe for the RPA assay was designed to allow assessment of both full length and exon 9-deleted (ΔE9) mRNA. As shown in **Fig. 3A,** both forms of CETP RNA are present in SW872 cells, the full length message represented by a 277 base protected fragment and the ΔE9 CETP message represented by 217 base protected fragment. The mRNA level of ΔE9 is reported to vary from 14 to 46% of total CETP mRNA depending on the tissue (18). In the SW872 cells we observed ΔE9 to be approximately 30% of total mRNA. Both the full length and ΔE9 CETP mRNA levels increased in response to cholesterol (Fig. 3B). In repeated experiments, LDL cholesterol increased CETP mRNA approximately 2-fold and 25-OH cholesterol by ~75% (Fig. 3C). These

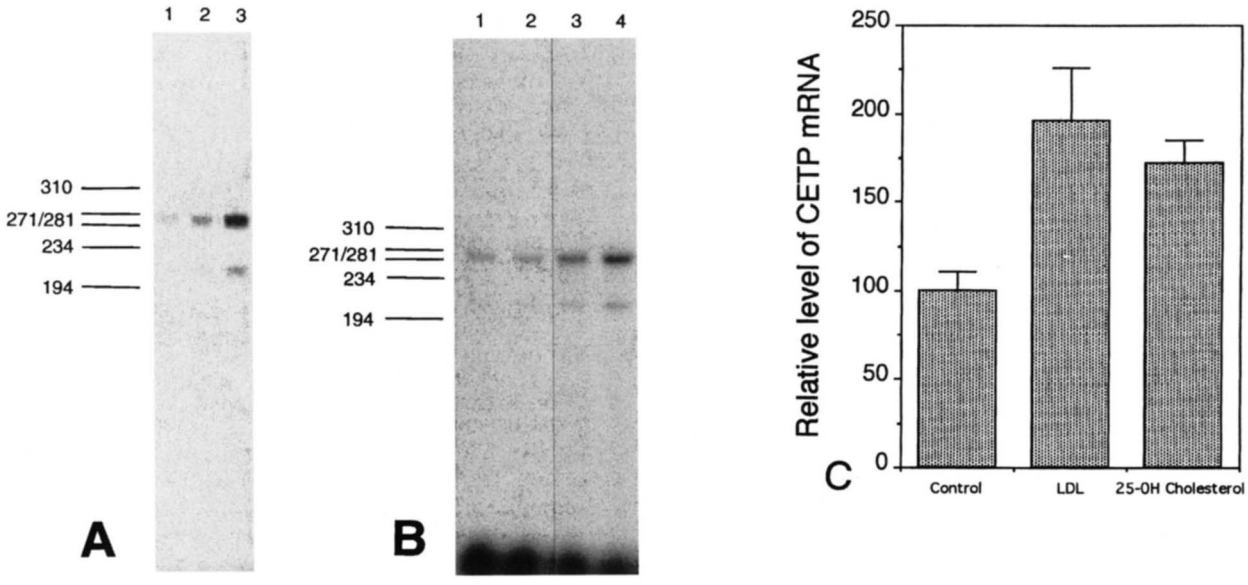

Fig. 3. Determination of CETP mRNA levels by ribonuclease protection assay (RPA). Total RNA isolation and polyacrylamide gel electrophoresis was performed as described under Methods and Materials. The 3′ antisense CETP riboprobe protected a 277 base fragment corresponding to the wild type CETP mRNA and a 217 bp fragment corresponding to the ΔE9 CETP mRNA. A) SW872 cells were grown to confluence as described under Methods and Materials and total RNA was isolated and used in RPA. Lane 1, 5 μg RNA; lane 2, 10 μg RNA; lane 3, 15 μg RNA. B) SW872 cells were grown to confluence as described under Methods and Materials and treated with 4 μg/ml 25-OH cholesterol in ethanol overnight followed by RNA isolation and analysis. Control cells were treated with equivalent levels of ethanol as in treated cultures. Ten μg total RNA was used in each RPA. Lane 1–2, control; lane 3, 4 μg/ml 25-OH cholesterol treated; lane 4, 8 μg/ml 25-OH cholesterol treated. C) Cells were treated with 100 μg/ml LDL or 8 μg/ml 25-OH cholesterol and the levels of CETP mRNA were determined as above. The results are the mean ± SEM of four independent determinations.

data suggest that a primary effect of cholesterol on increasing CETP levels in the liposarcoma cells is at the mRNA level.

It has been speculated (18) that the amount of CETP secreted from a cell can be controlled by changes in the ΔE9 CETP variant, which is poorly secreted by cells due to retention within the endoplasmic reticulum. This does not appear to be a control mechanism in the SW872 cells, however, as no obvious change in the ratio of ΔE9 to total CETP mRNA was observed. The levels of ΔE9 remained at ~30% regardless of treatment (mean = 29.2 ± 0.66, n = 27).

In summary, we have identified regulated expression and secretion of functional CETP in the human liposarcoma cell line SW872. While CETP expression has been previously reported in cell culture, the very low activities and lack of published data demonstrating cholesterol regulation have precluded use of cells such as HepG2 to study details of regulation and secretion. While it is not clear that this adipocyte-derived cell line will provide a predictive model for all cell types, it should nonetheless provide a useful tool for understanding the regulation and secretion of this important lipoprotein.

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