Novel mechanistic insight in cholesteryl ester transfer protein production and pharmacological inhibition

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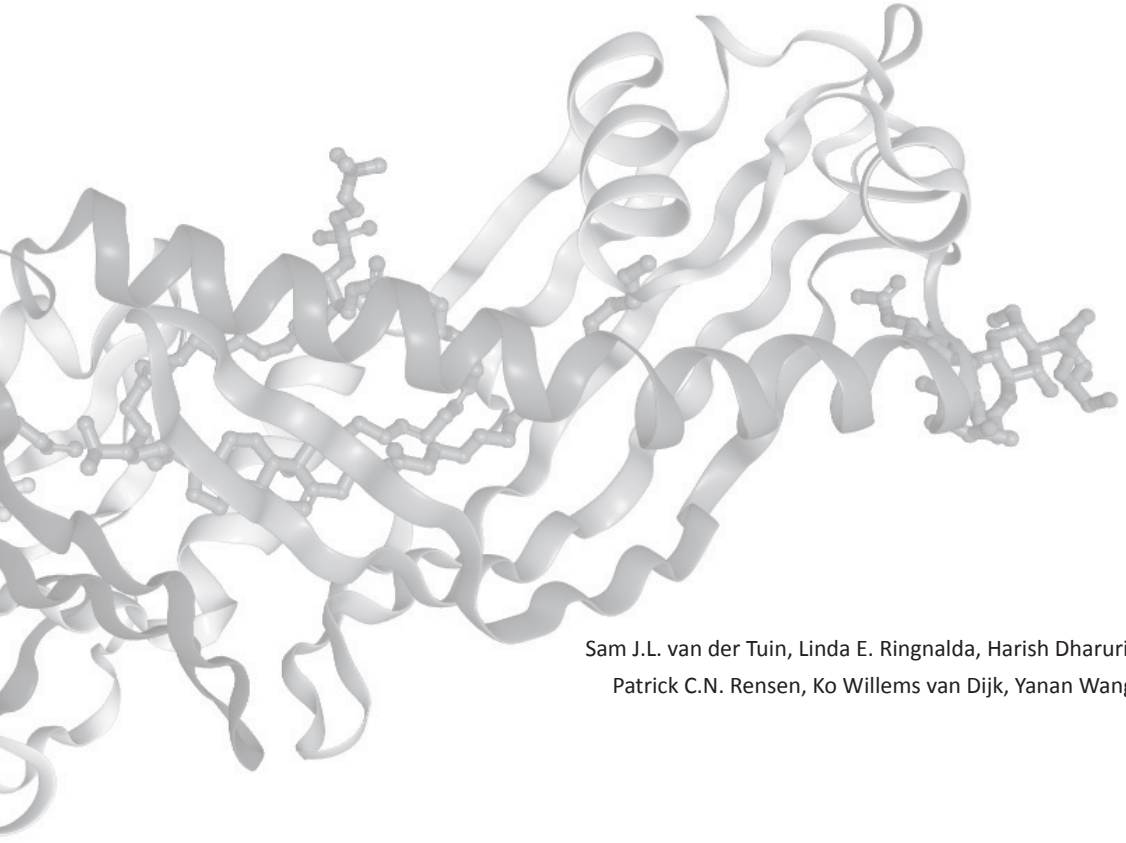
Chapter 3

Hepatic expression of cholesteryl ester transfer protein is confined to F4/80^{+}Ly6C^{-}Clec4f^{+}Vsig4^{+} macrophages



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ABSTRACT

Recently, we have shown that hepatic macrophages are the principal source of plasma CETP both in humans and human CETP transgenic mice. However, only a sub fraction of those macrophages express CETP. In the current study, we aimed to elucidate the subtype of hepatic macrophages that express CETP. Microarray analysis was performed on livers of APOE-3*Leiden.CETP mice at several time points after depletion of hepatic macrophages by liposomal clodronate. Clodronate largely reduced hepatic F4/80^{+} cells (-57%), CETP (-97%) and resident mature macrophages (Clec4f: -99%; Vsig4: -99%), but not immature macrophages (Ly6C: -0%). The re-appearance of CETP expression in the liver after clodronate treatment coincided with the re-appearance of Clec4f and Vsig4. Immunohistochemical analysis showed that CETP co-localized with Clec4f^{+} resident mature macrophages and not with Ly6C^{+} immature macrophages in the liver. Our data clearly indicate that in the liver, CETP is exclusively expressed by F4/80^{+}Ly6C^{-}Clec4f^{+}Vsig4^{+} macrophages that likely represent resident rather than immature macrophages.

INTRODUCTION

Cholesteryl ester transfer protein (CETP) is a plasma protein that plays a pivotal role in the metabolism of high density lipoprotein (HDL) and (very) low density lipoproteins [(V)LDL].[1] Genetic deficiency for CETP increases plasma HDL-cholesterol (C) and small-molecule CETP inhibitors induce a beneficial lipoprotein profile including reduced VLDL-C and triglycerides (TG) and raised HDL-C.[2-6] In addition, we previously showed that the CETP inhibitor anacetrapib dose-dependently reduced atherosclerosis development in APOE*3-Leiden. CETP mice, a mouse model for human-like lipoprotein metabolism that expresses human CETP under control of its natural flanking regions,[5] corroborating that CETP inhibition is a potential strategy for the prevention of cardiovascular disease.

Notably, *CETP* mRNA is found in several mammalian species, including humans and rabbits, but not mice and rats. In humans, the tissue(s) and cell type(s) contributing to the plasma CETP pool have been obscure until we recently demonstrated that the majority of plasma CETP is derived from hepatic macrophages (i.e. Kupffer cells).[7] We showed that hepatic expression of *CETP* is associated with that of macrophage markers (e.g. *CD68* and *MARCO*), and that *CETP* mRNA and protein co-localize with those macrophage markers in humans. In addition, we observed that elimination of hepatic macrophages [F4/80 positive (F4/80⁺) cells] in APOE*3-Leiden.CETP mice virtually abolished hepatic *CETP* mRNA and protein expression and largely reduced the plasma CETP concentration. Remarkably, immunofluorescent staining revealed that, in livers of both humans and APOE*3-Leiden. CETP mice, only a subtype of F4/80⁺ macrophages expresses CETP.

It should be noted that F4/80 is a general marker for monocytes and macrophages including, but not restricted to, Kupffer cells. Kupffer cells are resident mature macrophages that reside in the sinusoids of the liver and represent one of the largest macrophage populations in the body. [8] Experimental studies have revealed that Kupffer cells are a heterogeneous population of immune cells that fulfil diverse functions in homeostasis[9-11] and inflammation.[12-14] Traditionally, it was thought that Kupffer cells are not self-renewing and are replenished from bone-marrow derived monocytes.[10, 15, 16] However, recently is has been proposed that Kupffer cells are a self-renewing population and divide as mature cells, or originate from an intra-hepatic progenitor cell pool.[17-19]

In the current study, we aimed to elucidate which subtype(s) of F4/80⁺ cells expresses CETP. Firstly, a microarray analysis was performed on livers obtained from APOE*3-Leiden. CETP mice, treated with liposomal clodronate, resulting in virtually abolished hepatic CETP expression.[7] Next, we performed a study to assess the time course of re-appearance of CETP expression in the liver upon liposomal clodronate treatment, and simultaneously the dynamics of mRNA and protein markers for various macrophage sub populations.

MATERIALS AND METHODS

Animals and diet

Female APOE*3-Leiden.CETP transgenic mice[20] were housed under standard conditions with a 12 h: 12 h light-dark cycle and had free access to food and water during the experiment. Body weight and food intake were monitored during the study. Mice were fed a semi-synthetic cholesterol-rich diet, containing 15% (w/w) cacao butter, 1% (w/w) corn oil and 0.1% cholesterol (Western-type diet; AB-Diets) for a run-in period of 6 weeks. After randomization according to plasma total cholesterol (TC), HDL-C, triglycerides, body weight and age, ensuring that all mice were equally old when they were sacrificed, mice received two intraperitoneal injections of 4 mL/kg bodyweight liposomal clodronate (20 mg/kg bodyweight; purchased from Dr. N. van Rooijen, Amsterdam) at a 3-day interval to deplete macrophages from the liver.[21] Mice were terminated 3 days, or 3, 6 or 9 weeks after the second injection. Control mice received no liposomal clodronate treatment. All animals were killed by CO_2 inhalation. The Institutional Ethics Committee for Animal Procedures from the Leiden University Medical Centre, Leiden, The Netherlands, approved the protocol.

RNA isolation, cDNA synthesis and real time qPCR

Livers were isolated, and total RNA was extracted from pieces using the Nucleospin RNAII kit (Macherey-Nagel) according to the manufacturer's protocol. RNA concentration was determined by Nanodrop technology (Thermo Scientific). Total RNA was reverse-transcribed with iScript cDNA synthesis kit (Bio-Rad) and qPCR was performed using a CFX96™ (Bio-Rad). Gene expression was normalized to beta-2 microglobulin, hypoxanthine-guanine phosphoribosyltransferase and beta-actin. Relative expression was calculated and normalized to control group using Bio-Rad CFX Manager™ software 3.0 (Bio-Rad). Primer sequences can be found in Table S1. Microarrays, including RNA labelling, hybridization, data extraction and quality control, were performed by ServiceXS B.V. (Leiden, The Netherlands).

Table S1: Primer sequences used for qPCR

Gene	Forward primer	Reverse Primer
ß-actin	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
ß-2m	TGACCGGCTTGTATGCTATC	CAGTGTGAGCCAGGATATAG
CETP	CATGTCTCGGCTCGAGGTAG	TTCTGCTACAAGCCCCATCC
Clec4f	ACTGAAGTACCAAATGGACAATGTTAGT	GTCAGCATTCACATCCTCCAGA
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Hprt	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG
Ly6C	CTGCAACCTTGTCTGAGAGGA	GTCCCTGAGCTCTTTCTGCAC
Vsig4	TCACCTATGGCCACCCCACC	AGGCGGCCTCTGTACTTTGCCT

ß-actin, Beta-actin; ß-2m, ß-2 microglobulin; *CETP*, cholesteryl ester transfer protein; *Clec4f*, C-type lectin domain family 4, member f; *Hprt*, hypoxanthine ribosyltransferase; *Vsig4*, V-set and immunoglobulin domain containing 4.

Immuno-histochemical and -fluorescence staining

Paraffin-embedded sections of APOE*3-Leiden.CETP mouse livers (5 µm) were stained for F4/80 and human CETP (ab51771; 1/1000, Abcam) as described previously,[7] as well as Clec4f (MAB2784; 1/1000, R&D Systems), Vsig4 (AF4674; 1:1000, R&D Systems) and Ly6C (ab15627; 1/400, Abcam) For immunofluorescence staining the secondary antibodies donkey anti-rabbit Alexa488 (A21206; Invitrogen) and goat anti-rat Alexa555 (A21434; Invitrogen) were used. Finally tissue sections were mounted with VECTASHIELD® Mounting Medium with DAPI (Vector Laboratories). Positive cells were counted using a LeicaCTR5500 fluorescence microscope (Leica Microsystems GmbH).

Plasma lipid analysis

Plasma total cholesterol and triglycerides were assayed using the commercially available enzymatic kits 236691, 11488872 (Roche Molecular Biochemicals), respectively. To measure HDL-C, apoB-containing particles were precipitated from plasma with 20% polyethylene glycol 6000 in 200 mM glycine buffer (pH 10) and cholesterol was measured in the supernatant.[5]

Statistical analysis

Significance of differences between the groups was calculated non-parametrically using a Mann-Whitney U-test for independent samples. All groups were compared to the control group (t=0 without an injection of liposomal clodronate). Bonferroni-Holm's method was used to determine the level of significance in case of multiple comparisons. For correlation, a linear regression analysis was performed. SPSS 18.0 for Windows was used for statistical analysis. Values are presented as mean±SD. P-values <0.05 were considered statistically significant for single comparison.

RESULTS

Liposomal clodronate treatment depletes the liver from tissue resident mature macrophages and CETP

Previously, we showed that 3 days after liposomal clodronate treatment of APOE*3-Leiden. CETP mice, the general monocyte/macrophage marker F4/80 was largely decreased in the liver, with respect to mRNA expression (-88%) and the number of protein-positive cells (-74%). Concomitantly, clodronate virtually abolished hepatic *CETP* mRNA (-96%) as well as CETP^{+} cells (-96%).[7] To better understand which macrophage subset expresses CETP, we performed microarray analysis on livers from these clodronate-treated and control mice (n=4 per group). Clodronate downregulated 214 genes and upregulated 40 genes to be up

regulated (false discovery rate P<0.05; Table S2). Pathway analysis showed that clodronate significantly regulated immune-associated pathways (Table S3).

The two most significantly (down)regulated genes were V-set and immunoglobulin domain containing 4 (*Vsig4*) and C-type lectin domain family 4, member f (*Clec4f*), both of which are markers of tissue resident mature macrophages.[12, 22, 23] Clodronate did not affect expression of lymphocyte antigen 6C (*Ly6C*) and Macrophage-1 antigen (*Mac-1*), which are markers of infiltrating immature monocytes/macrophages.[24] Expression of these macrophage marker genes were validated by qPCR (Fig. 1). Liposomal clodronate almost completely abolished the expression of *Clec4f* (-99%, P<0.001) and *Vsig4* (-98%, P<0.001), without affecting the expression of *Ly6C* and *Mac-1* (NS). Since clodronate abolished *CETP* expression (-96%)[7] similarly to *Clec4f* and *Vsig4*, these data suggest that *CETP* is expressed by tissue resident mature macrophages.

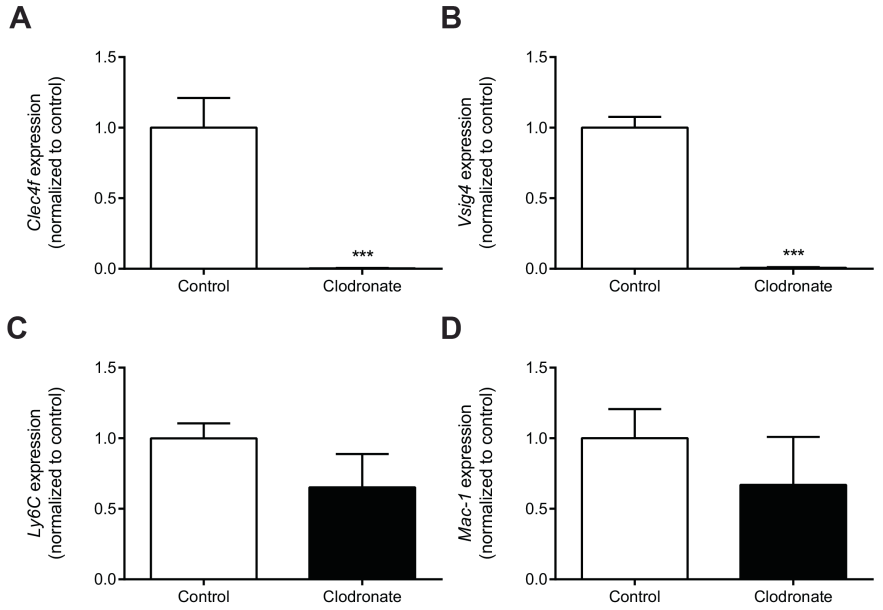

Figure 1. Liposomal clodronate treatment abolishes hepatic mRNA expression of *Clec4f* and *Vsig4* without affecting *Ly6C* and *Mac-1*

Female APOE*3-Leiden.CETP mice fed a Western-type diet were treated without or with liposomal clodronate. After 3 days, livers were assayed for *Clec4f* (A), *Vsig4* (B), *Ly6C* (C) and *Mac-1* (D) mRNA expression.

Data are presented as mean±SD. Student's T-test; ***P<0.001 compared with the control group.

Reappearance of hepatic CETP expression after liposomal clodronate treatment coincides with reappearance of hepatic Clecf4 and Vsig4 expression

We reasoned that if CETP is indeed expressed by mature macrophages, reappearance of CETP in the liver should parallel the reappearance of Clec4f and Vsig4. Therefore, a time-course study was performed in which mice were sacrificed 3 days (t=0), 3 weeks, 6 weeks or 9 weeks after clodronate treatment. In line with our previous study,[7] liposomal clodronate largely decreased *F4/80* mRNA (-82%; Fig. 2A) and F4/80$^+$ cells (-57%; Fig. 2B), and virtually depleted the liver from *CETP* mRNA (-92%; Fig. 2C) and CETP-positive (CETP$^+$) cells (-97%; Fig. 2D). *F4/80* mRNA and F4/80$^+$ cells were restored already 3-6 weeks after clodronate treatment (Figs. 2A, B). In contrast, *CETP* mRNA and CETP$^+$ cells gradually returned to baseline levels and were normalized only after 9 weeks of clodronate treatment (Figs. 2C, D) Notably, clodronate had virtually no effect on *Ly6C* mRNA (-28%; Fig. 2E) and Ly6C$^+$ cells (-0%; Fig. 2F) just after injections and during the recovery period. Interestingly, the immediate effect of clodronate treatment on Clec4f mRNA and positive cells (Fig. 2G and H, respectively) and Vsig4 mRNA and positive cells (Fig. 2I and J, respectively) paralleled those of CETP mRNA and positive cells, albeit that at 9 weeks Clec4f$^+$ cells and Vsig4$^+$ cells were still slightly decreased compered to control mice.

Next, we performed correlation analyses between the hepatic expression of *CETP* with those of *F4/80* (Fig. 3A), *Ly6C* (Fig. 3C), *Clec4f* (Fig. 3E), and *Vsig4* (Fig. 3G). *CETP* expression significantly correlated with *F4/80*, *Clec4f* and *Vsig4* (all P<0.001) and *Ly6C* (P<0.05), in the following order: *Clec4f* (R^2=0.793) > *Vsig4* (R^2=0.585) > *F4/80* (R^2=0.464) > *Ly6C* (R^2=0.135). These data were paralleled by correlations between hepatic CETP$^+$ cell number with hepatic cell number positive for F4/80 (Fig. 3B), Ly6C (Fig. 3D), Clec4f (Fig. 3F), and Vsig4 (Fig. 3H). CETP$^+$ cell number significantly correlated with the number of cells positive for F4/80, Clec4f and Vsig4 (all P<0.001), but not Ly6C (n.s.), in the following order: Clec4f (R^2=0.879) > Vsig4 (R^2=0.779) > F4/80 (R^2=0.690) > Ly6C (R^2=0.008).

CETP is present in hepatic Clec4f-positive but not Ly6C-positive cells

Since the very strong correlations observed between hepatic CETP and Clec4f/Vsig4 (expression and number of positive cells) suggested co-localization, we performed double immunofluorescence staining of CETP and Clec4f on liver sections. Due to technical limitations, it was not possible to perform CETP and Vsig4 double immunofluorescence staining. Hepatic CETP co-localized with F4/80 expression, but only 35% of F4/80$^+$ cells were CETP$^+$ (Fig. 4A), which is in agreement with our previous findings.[7] Ly6C$^+$ cells did not stain for CETP (Fig. 4B), while all Clec4f$^+$ cells stained positive for CETP (Fig. 4C).

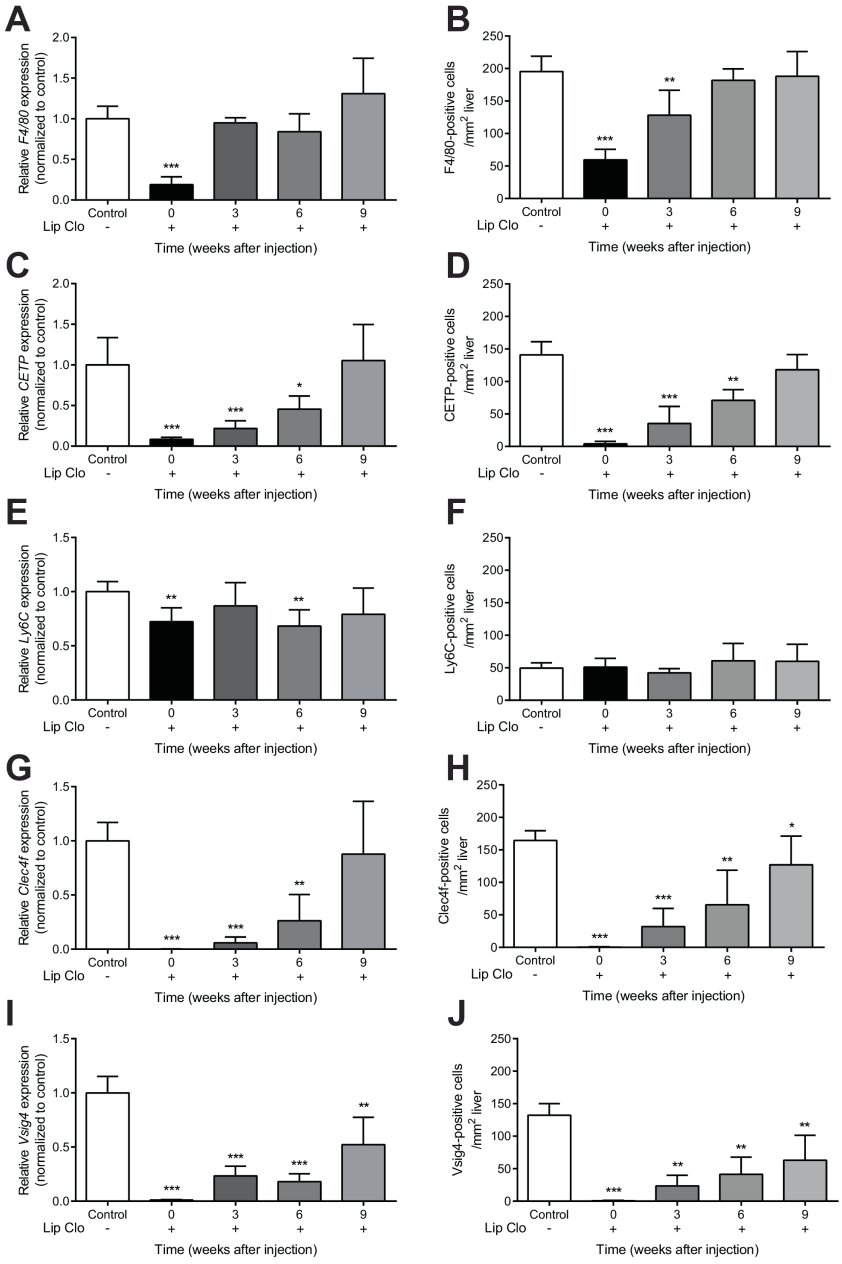

Figure 2. The time course of re-appearance of hepatic CETP expression and positive cells following liposomal clodronate treatment coincides with those of Clec4f and Vsig4 but not Ly6C

Female APOE*3-Leiden.CETP mice were fed a Western-type diet and sacrificed 3 days (t=0), 3, 6 and 9 weeks after liposomal clodronate (Lip Clo) treatment, and untreated mice were taken along as control. Livers were assayed for mRNA (A, C, E, G and I) and protein (B, D, F, H and J) of F4/80 (A, B), CETP (C, D), Ly6C (E, F), Clec4f (G, H) and Vsig4 (I, J).

Data are presented as mean±SD. *P<0.05, **P<0.01, ***P<0.001 compared with the control group.

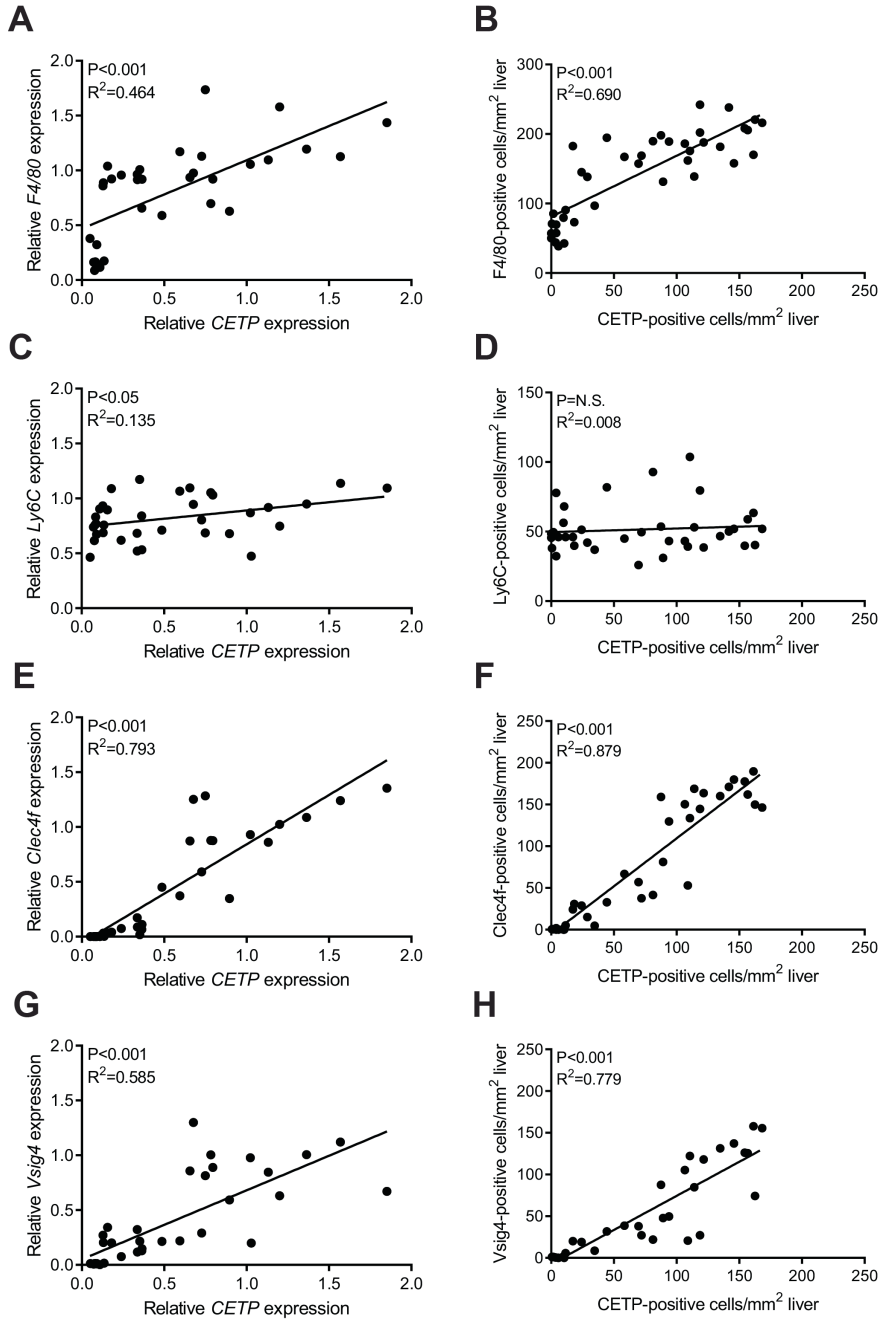

Figure 3. Hepatic CETP mRNA and positive cells strongly correlate with those of Clec4f and Vsig4, but not Ly6C

Linear regression analyses were performed on the correlations between hepatic CETP mRNA (A, C, E, G) or protein (B, D, F, H) with F4/80 (A, B), Ly6C (C, D), Clec4f (E, F) and Vsig4 (G, H).

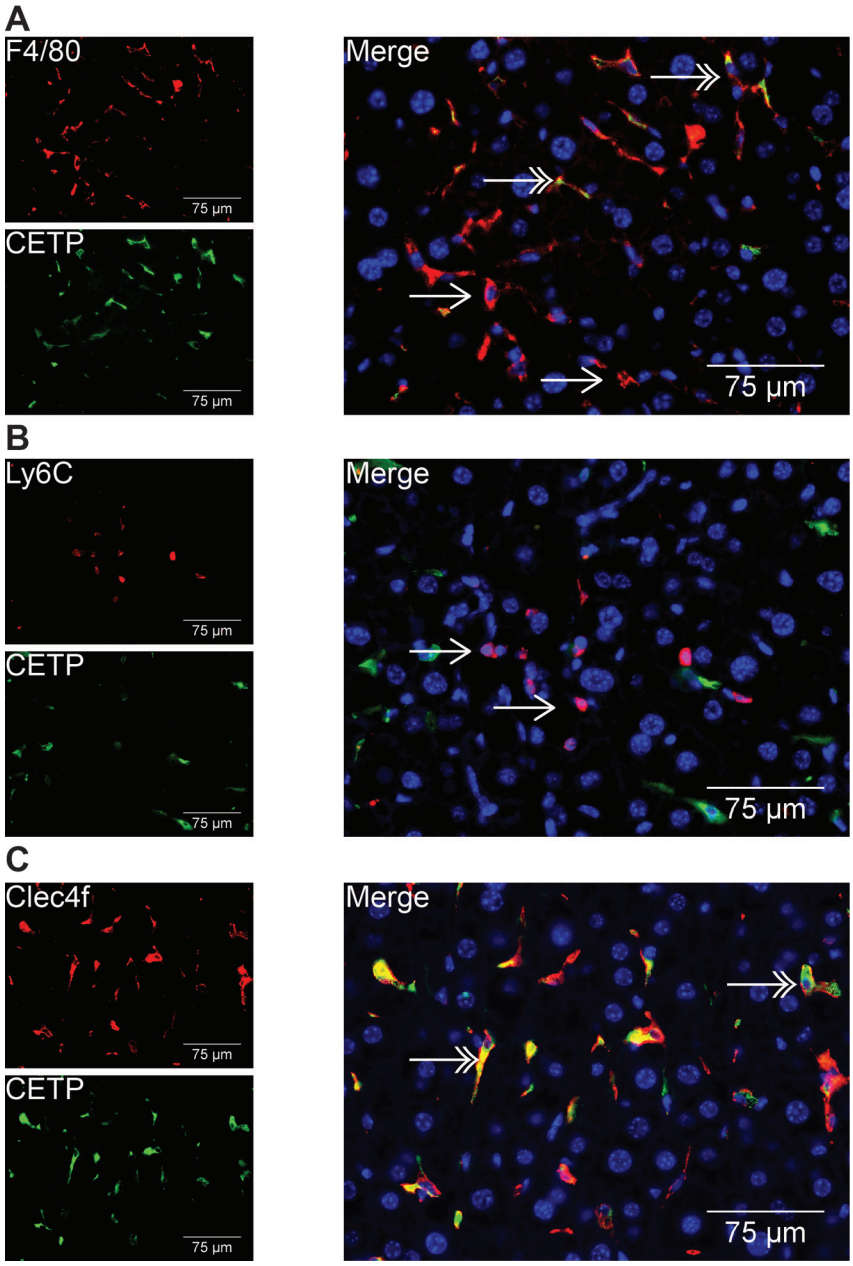

Figure 4. CETP protein is not co-localized with Ly6C protein, but does co-localizes with Clec4f protein
Livers of non-injected female APOE*3-Leiden-CETP mice were assayed for co-localization of CETP and F4/80 (A), Ly6C (B) and Clec4f (C). Red; F4/80, Ly6C or Clec4f, Green; CETP, Blue; DAPI. Double arrows indicate co-localization of macrophage markers and CETP. Single arrows indicate macrophage markers that do not co-localize with CETP.

Liposomal clodronate treatment transiently increases HDL-cholesterol

To evaluate the relevance of the expression of CETP by hepatic mature macrophages for plasma cholesterol metabolism, plasma cholesterol (Fig. 5A), (V)LDL-cholesterol (Fig. 5B) and HDL-cholesterol (Fig. 5C) were measured at the several time-points after clodronate treatment. The transient decrease in plasma CETP was paralleled by a transient increase in HDL-C (data not shown).

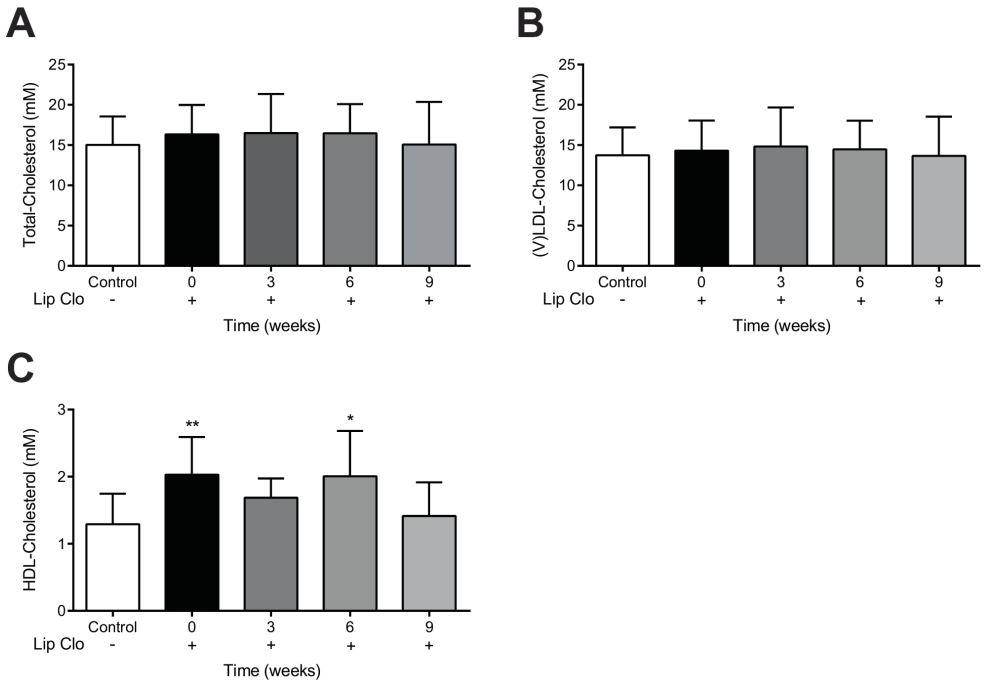

Figure 5. Liposomal clodronate treatment transiently increases plasma HDL-cholesterol
Female APOE*3-Leiden.CETP mice were fed a Western-type diet and sacrificed 3 days (t=0), 3, 6 and 9 weeks after liposomal clodronate (Lip Clo) treatment, and untreated mice were taken along as control. Plasma was assayed for A) total cholesterol, B) (V)LDL-cholesterol and C) HDL-cholesterol.
Data are presented as mean±SD. *P<0.05, **P<0.01 compared with the control group.

DISCUSSION

Recently, we have shown that macrophages in the liver are the primary source of plasma CETP. In addition, we observed that 39% of $CD68^+$ macrophages in the liver co-express CETP in humans, and 57% of $F4/80^+$ macrophages in the liver co-express CETP in human CETP transgenic mice.[7] In this study we set out to evaluate which macrophage population(s) express(es) CETP by evaluation the time course of repopulation of the liver with

macrophages following liposomal clodronate-induced macrophage elimination in APOE*3-Leiden.CETP transgenic mice. Co-expression and co-localization studies clearly demonstrate that CETP is expressed by F4/80$^+$Ly6C$^-$Clec4f$^+$Vsig4$^+$ macrophages in the liver, but not by F4/80$^+$Ly6C$^+$Clec4f$^-$Vsig4$^-$ macrophages.

Injection of liposomal clodronate is a well-established method to deplete the liver from F4/80$^+$ macrophages without damaging other cell types, including hepatocytes and hepatic stellate cells[25] and without induction of the production of pro-inflammatory cytokines and/or nitric oxide.[26] We confirmed that 3 days after liposomal clodronate injection, hepatic mRNA and protein expression of F4/80 was largely decreased, followed by the progressive repopulation of the liver with F4/80$^+$ cells to the baseline at 3-6 weeks. Liposomal clodronate completely depleted the liver from cells expressing Clec4f that has been termed the 'Kupffer cell marker'.[27, 28] In our study, the recovery of Clec4f$^+$ cells took approx. 9 weeks. Despite differences in liposomal clodronate injection protocols, previous studies showed a similar time of reappearance of F4/80$^+$ cells, varying from 2 weeks[19, 29] to 4 weeks.[23] For Clec4f$^+$ cells a much shorter turnover time was observed (4 weeks)[23] as compared with the present study, which may be due to the differences in the liposomal clodronate injection protocol. We injected mice twice with a 3-day interval as compared to once.[23] Our findings clearly show that Clec4f$^+$ cells take a longer time to reappear than F4/80$^+$ cells, most probably because F4/80$^+$Clec4f$^+$ cells are more mature macrophages.

We observed that F4/80$^+$Ly6C$^-$Clec4f$^+$Vsig4$^+$ macrophages, but not Ly6C$^+$ macrophages, express CETP, indicating that only macrophages in a specific maturation state express *CETP*. Since we did not detect CETP expression in macrophages of adipose tissue,[7] liver-specific factors are probably needed for F4/80$^+$Ly6C$^-$Clec4f$^+$Vsig4$^+$ macrophages to express CETP. It has been reported that the CETP gene contains responsive elements for both liver-X-receptor α (*LXRα*)[30] and farnesoid-X-receptor (*FXR*).[31] The *LXRα* is expressed in multiple organs, while the expression of the FXR is confined to the liver and the intestine.[32] In the liver, oxysterols activate LXRα; while, bile acids produced by hepatocytes, are the natural ligand for the FXR. Administration of an LXRα agonist increases hepatic expression of *CETP* as well as the plasma CETP concentration in CETP transgenic mice.[33] In addition, treatment of APOE*3-Leiden.CETP mice with the bile acid taurocholic acid also increases hepatic *CETP* expression and plasma CETP concentration.[31] Collectively, the presence of both LXRα and FXR signalling in liver may be crucial for the specific capacity of hepatic F4/80$^+$Ly6C$^-$Clec4f$^+$Vsig4$^+$ macrophages to secrete CETP.

The observation that F4/80$^+$Ly6C$^-$Clec4f$^+$Vsig4$^+$ macrophages, being liver resident mature macrophages (i.e. Kupffer cells), secrete CETP, while other subtypes of macrophage do not, is likely of biological significance. Kupffer cells play a pivotal role in the first defence, the innate immune response, to bacteria and other pathogens coming from the intestines via the portal vein to the liver.[34, 35] Lipopolysaccharide (LPS), a major outer membrane

constituent of Gram-negative bacteria, is a potent endotoxin that activates Kupffer cells to reduce the toxicity of LPS. It has been shown LPS administration rapidly decreases hepatic *CETP* mRNA expression and plasma CETP concentration, accompanied by an increased HDL-C level. Taking into account the anti-inflammatory properties of HDL,[36, 37] reducing CETP expression upon Kupffer cells activation represents an adaptive response to preserve or increase HDL levels, thereby decreasing LPS toxicity, and increasing host survival. After LPS administration, mice expressing human CETP showed a remarkable enhanced survival rate as compared with wild-type mice deficient of CETP.[38] Apparently, the described capacity of CETP to induce binding of LPS to HDL[38] prevents hyper activation of the immune system in the initial response to LPS.

Here we describe F4/80^{+}Ly6C^{-}Clec4f^{+}Vsig4^{+} macrophages being the only cell type in the liver expressing CETP. In contrast to mouse, in humans *CLEC4F* expression is not confined to the liver,[39] while VSIG4 is exclusively expressed by macrophages in the liver.[22, 40] Here we show that Vsig4 paralleled the reappearance of Clec4f and CETP. In addition, correlation analysis from our previous study[7] revealed a strong correlation between *CETP* and *VSIG4*. In line with the current findings, CETP expression in humans seems also confined to Kupffer cells.

Traditionally, it was thought Kupffer cells are replenished from bone-marrow derived cells.[10, 15, 16] However, recently it has been proposed that Kupffer cells are a self-renewing population and divide as mature cells, or originate from an intra-hepatic progenitor cell.[17-19] Here we show liposomal clodronate completely abolished mRNA and protein expression of Kupffer cell markers Clec4f and Vsig4, suggesting that Kupffer cells are not replenished by division of mature Kupffer cells, because there are no mature Kupffer cells to divide. Unfortunately, because of the design of the study it cannot be concluded whether Kupffer cells are replenished from bone-marrow cells. The influx of monocytes precedes[19] the time-point we have examined in this study. Finally, whether Kupffer cells originate from an intra-hepatic progenitor cell cannot be concluded from this study.

In the present study, we revealed that in the liver, CETP is expressed by mature (i.e. F4/80^{+}Ly6C^{-}Clec4f^{+}) macrophages but not immature (i.e. Ly6C^{+}) macrophages. We propose that the regulation of CETP expression in resident mature macrophages is of importance for the function of Kupffer cells in mediating host defence. Under inflammatory conditions Kupffer cells lose their capability to express CETP, leading to increased HDL levels, which may be beneficial by attenuating inflammation and toxicity.

ACKNOWLEDGEMENTS

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SUPPLEMENTAL DATA

Table S2: Differentially expressed genes after liposomal clodronate treatment.

Gene Nama	Gene Symbol	Fold change	Adjusted P-value
V-set and immunoglobulin domain containing 4	*Vsig4*	-4345.074	2.85E-12
C-type lectin domain family 4, member f	*Clec4f*	-436.509	2.85E-12
Complement factor properdin	*Cfp*	-143.034	2.78E-04
Complement component 1, q subcomponent, C chain	*C1qc*	-120.794	5.73E-04
Complement component 1, q subcomponent, beta polypeptide	*C1qb*	-96.552	1.72E-03
Fc receptor, IgG, low affinity IV	*Fcgr4*	-41.328	2.76E-03
Folate receptor 2 (fetal)	*Folr2*	-36.310	2.86E-08
Latrophilin 1	*Lpl*	-26.591	1.35E-02
Syndecan 3	*Sdc3*	-24.433	1.69E-04
Interferon-induced protein with tetratricopeptide repeats 3	*Ifit3*	-18.883	2.72E-03
AXL receptor tyrosine kinase	*Axl*	-18.069	9.74E-05
Complement component 1, q subcomponent, alpha polypeptide	*C1qa*	-17.664	1.78E-06
Eosinophil-associated, ribonuclease A family, member 2	*Ear2*	-17.506	1.95E-04
Fc receptor, IgE, high affinity I, gamma polypeptide	*Fcer1g*	-14.941	6.13E-03
LIM domain only 2	*LOC100038882*	-12.908	7.81E-03
Lectin, galactoside-binding, soluble, 3 binding protein	*Lgmn*	-12.193	1.31E-03
Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	*Slc11a1*	-11.617	1.69E-04
Allograft inflammatory factor 1	*Aif1*	-11.393	2.83E-04
Chemokine (C-X-C motif) ligand 9	*Cxcl9*	-11.299	3.82E-03
Solute carrier family 15, member 3	*Slc15a3*	-11.023	3.93E-04
CD52 antigen	*Cd52*	-10.904	1.69E-04
Wfdc17	*OTTMUSG00000000971*	-10.607	1.24E-02
Cytochrome P450, family 8, subfamily b, polypeptide 1	*Cyp8b1*	-10.239	9.29E-04
TYRO protein tyrosine kinase binding protein	*Tyrobp*	-9.802	1.29E-04
Interferon, alpha-inducible protein 27	*Ifi27*	-9.552	2.40E-03
Lymphocyte antigen 6 complex, locus A	*Ly6a*	-9.017	1.70E-02
Erythrocyte protein band 4.1-like 3	*Epb4.1l3*	-8.819	9.86E-05
Ficolin A	*Fcna*	-8.729	7.20E-03
Eosinophil-associated, ribonuclease A family, member 4	*Ear4*	-8.537	8.19E-05
Complement component 6	*C6*	-8.201	8.77E-03
Kruppel-like factor 13	*Laptm5*	-7.962	1.66E-02
Mannose receptor, C type 1	*Ms4a6d*	-7.582	1.41E-02
Thymosin, beta 4, X chromosome	*Tmsb4x*	-7.383	1.96E-03
Phosphodiesterase 1B, Ca2+-calmodulin dependent	*Pdia3*	-7.038	5.24E-03
Signal-regulatory protein alpha	*Sirpa*	-6.097	8.20E-03
EGF-like module containing, mucin-like, hormone receptor-like sequence 1	*Emr1*	-6.044	1.69E-04
Transmembrane protein 86A	*Tmem86a*	-5.869	7.23E-03
ATPase, Na+/K+ transporting, beta 3 polypeptide	*Atp1b3*	-5.784	1.06E-04
CD86 antigen	*Cd86*	-5.587	1.29E-04
Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	*Gngt2*	-5.414	1.07E-03
Lymphocyte antigen 6 complex, locus A	*Ly86*	-5.404	3.61E-03
Cytochrome b-245, alpha polypeptide	*Cyba*	-5.331	6.79E-03
CD68 antigen	*Cd68*	-5.303	2.27E-02
Vascular cell adhesion molecule 1	*Vcam1*	-5.127	3.43E-02
Serine (or cysteine) peptidase inhibitor, clade A, member 3G	*Serpina3g*	-5.095	2.82E-02
Limb-bud and heart	Lgals3bp	-4.951	1.89E-03

Leucine rich repeat containing 33	*Lst1*	-4.850	9.86E-05
Lymphocyte antigen 9	*Maged1*	-4.479	1.35E-03
Tumor necrosis factor, alpha-induced protein 2	*Tnfaip2*	-4.416	2.37E-03
Guanylate binding protein 1	*Gbp1*	-4.250	8.40E-04
PREDICTED: similar to dendritic cell-associated C-type lectin-1; DECTIN-1	*LOC667370*	-4.200	7.85E-03
Placenta-specific 8	*Plac8*	-4.184	7.07E-03
purinergic receptor P2Y, G-protein coupled 13	*P2ry13*	-4.130	1.69E-04
PREDICTED: hypothetical protein LOC100047934	*LOC100048461*	-4.111	1.17E-04
Phospholipase D family, member 4	*Pld4*	-4.107	1.59E-04
Nudix (nucleoside diphosphate linked moiety X)-type motif 19	*Oas1g*	-3.996	4.97E-02
2'-5' oligoadenylate synthetase-like 1	*Oasl2*	-3.936	2.08E-02
Claudin 1	*Cldn1*	-3.848	1.24E-02
C-type lectin domain family 7, member a	*Clec7a*	-3.816	1.68E-03
Creatine kinase, brain	*Ckb*	-3.807	1.15E-02
IQ motif containing GTPase activating protein 1	*Irf7*	-3.772	1.59E-04
Moesin	*Nckap1l*	-3.737	7.69E-03
Coactosin-like 1 (Dictyostelium)	*Cotl1*	-3.582	2.65E-03
TSC22 domain family, member 1	*Tsc22d1*	-3.574	1.79E-02
C-type lectin domain family 4, member a3	*Clec4a3*	-3.567	3.73E-04
RIKEN cDNA 4732429D16 gene	*4732429D16Rik*	-3.519	1.70E-05
Interferon gamma inducible protein 30	*Ifi30*	-3.498	1.76E-03
2'-5' oligoadenylate synthetase 2	*Oasl1*	-3.464	5.49E-03
Heme oxygenase (decycling) 1	*Hmox1*	-3.442	1.23E-02
Ecotropic viral integration site 2a	*Evi2a*	-3.438	1.70E-03
Formyl peptide receptor 2	*Fpr2*	-3.398	7.95E-03
Hexokinase 3	*Hk3*	-3.225	4.18E-03
DEXH (Asp-Glu-X-His) box polypeptide 58	*Dhx58*	-3.222	1.88E-02
2'-5' oligoadenylate synthetase 1G	*Oas2*	-3.168	3.12E-03
Syndecan binding protein (syntenin) 2	*Sdcbp*	-3.102	8.11E-04
Interleukin enhancer binding factor 3	*Iqgap1*	-3.054	3.28E-03
Colony stimulating factor 1 receptor	*Csf1r*	-3.039	5.06E-03
CD83 antigen	*Cd83*	-3.024	8.13E-03
Unc-93 homolog B1 (C. elegans)	*Unc93b1*	-3.000	1.02E-02
Fc receptor, IgG, low affinity III	*Fcgr3*	-2.952	5.77E-04
RAB8B, member RAS oncogene family	*Rab8b*	-2.950	5.30E-03
Glycolipid transfer protein	*Gltp*	-2.902	1.35E-03
Histocompatibility 2, class II, locus Dma	*H2-DMa*	-2.877	3.73E-03
CD97 antigen	*Cd97*	-2.876	6.81E-03
Eukaryotic translation initiation factor 2-alpha kinase 2	*Eif2ak2*	-2.872	1.35E-03
Chemokine (C-C motif) receptor 5	*Ccr5*	-2.716	1.27E-04
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	*Apobec1*	-2.700	3.18E-02
Serine (or cysteine) peptidase inhibitor, clade B, member 6a	*Serpinb6a*	-2.654	2.27E-02
Melanoma antigen, family D, 1	*Marcks*	-2.642	5.40E-03
RIKEN cDNA A430084P05 gene	*A430084P05Rik*	-2.631	3.26E-05
FYN binding protein	*Fyb*	-2.630	4.18E-03
Peripheral myelin protein 22	*Pmp22*	-2.586	9.90E-04
Matrix metallopeptidase 12	*Mrc1*	-2.575	1.20E-02
Vav 1 oncogene	*Vav1*	-2.566	3.99E-03
Phospholipase A2, group XV	*Pla2g15*	-2.563	4.52E-03
CD14 antigen	*Cd14*	-2.546	1.23E-02
Abl-interactor 3	*Abi3*	-2.452	1.54E-02
Chemokine (C-C motif) ligand 24	*Ccl24*	-2.449	1.69E-04
Toll-like receptor 2	*Tlr2*	-2.448	4.33E-03
MARCKS-like 1	*Marco*	-2.424	8.52E-03

N-acylsphingosine amidohydrolase 1	*Asah1*	-2.417	1.18E-03
Low density lipoprotein-related protein 12	*Lrrc33*	-2.411	5.21E-03
RIKEN cDNA 2310005E10 gene	*2310005E10Rik*	-2.383	1.44E-03
CD84 antigen	*Cd84*	-2.382	4.45E-03
cDNA sequence BC013712	*BC013712*	-2.373	1.87E-04
Serine (or cysteine) peptidase inhibitor, clade A, member 3F	*Serpina3f*	-2.368	2.57E-02
Rho GTPase activating protein 30	*Arhgap30*	-2.357	1.96E-03
Protein disulfide isomerase associated 3	*Pdlim4*	-2.355	1.27E-04
Tubulin, alpha 1B	*Tuba1b*	-2.347	1.24E-02
Sialic acid binding Ig-like lectin 1, sialoadhesin	*Siglec1*	-2.314	3.73E-04
Adrenergic receptor kinase, beta 1	*Adrbk1*	-2.303	2.02E-03
Arrestin, beta 2	*Arrb2*	-2.294	9.90E-04
Rho, GDP dissociation inhibitor (GDI) beta	*Arhgdib*	-2.284	3.22E-02
2'-5' oligoadenylate synthetase-like 2	*Obrgrp*	-2.280	9.84E-03
Leukocyte immunoglobulin-like receptor, subfamily B, member 4	*Lmo2*	-2.259	4.10E-02
DEAD (Asp-Glu-Ala-Asp) box polypeptide 24	*Ddx24*	-2.259	1.58E-03
cDNA sequence BC006779	*BC006779*	-2.249	3.47E-02
GTPase activating protein (SH3 domain) binding protein 2	*G3bp2*	-2.248	3.24E-02
Chemokine (C-C motif) ligand 6	*Ccl6*	-2.248	5.21E-03
SLAM family member 9	*Slamf9*	-2.229	1.73E-03
Expressed sequence AI451617	*AI451617*	-2.209	5.71E-03
PREDICTED: hypothetical protein LOC100038882	*LOC100047934*	-2.199	4.33E-03
RIKEN cDNA 2310016C08 gene	*2310016C08Rik*	-2.188	4.67E-03
FYVE, RhoGEF and PH domain containing 2	*Fgd2*	-2.174	9.86E-05
Lysosomal-associated protein transmembrane 5	*Lbh*	-2.171	4.93E-02
Triggering receptor expressed on myeloid cells-like 4	*Treml4*	-2.161	7.81E-03
Pleckstrin homology domain-containing, family A (phosphoinositide binding specific) member 2	*Plekha2*	-2.154	1.01E-02
RAS guanyl releasing protein 1	*Rasgrp1*	-2.152	2.08E-03
Protein tyrosine phosphatase, non-receptor type 6	*Ptpn6*	-2.150	2.08E-02
Phosphoglycerate mutase 1	*Pilrb1*	-2.137	5.25E-03
Scotin gene	*Scotin*	-2.126	3.47E-02
Phosphoenolpyruvate carboxykinase 1, cytosolic	*Pcolce2*	-2.111	1.44E-03
Interferon-induced protein 35	*Ifi35*	-2.098	2.67E-02
Josephin domain containing 1	*Kctd12*	-2.095	9.84E-03
Aldehyde dehydrogenase 3 family, member B1	*Aldh3b1*	-2.090	1.14E-03
Hstocompatibility 2, M region locus 3	*H2-M3*	-2.089	5.49E-03
Transmembrane emp24-like trafficking protein 10 (yeast)	*Tmed10*	-2.082	9.58E-03
Membrane-spanning 4-domains, subfamily A, member 8A	*Msn*	-2.072	3.24E-02
Serine/threonine kinase 17b (apoptosis-inducing)	*Stk17b*	-2.071	1.18E-03
Protein kinase C, delta binding protein	*Prkcd*	-2.070	4.39E-02
DNA segment, Chr 14, ERATO Doi 668, expressed	*D14Ertd668e*	-2.052	1.24E-02
cDNA sequence BC004728	*BC004728*	-2.013	4.72E-02
T-cell immunoglobulin and mucin domain containing 4	*Timd4*	-2.010	4.19E-03
Protein kinase C binding protein 1	*Prkcb*	-2.006	1.96E-03
C-type lectin domain family 4, member n	*Clec4n*	-2.006	3.24E-02
Interleukin 17 receptor C	*Il1a*	-2.005	7.38E-04
BTB (POZ) domain containing 11	*Btbd11*	-1.992	4.22E-03
Docking protein 2	*Dok2*	-1.979	2.30E-02
CD44 antigen	*Cd44*	-1.979	2.87E-02
Regulating synaptic membrane exocytosis 3	*Rims3*	-1.962	5.21E-04
Ral guanine nucleotide dissociation stimulator,-like 1	*Rgl1*	-1.959	3.68E-04
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	*Slc7a8*	-1.955	3.73E-04
CKLF-like MARVEL transmembrane domain containing 7	*Cmtm7*	-1.946	3.82E-03

EH-domain containing 4	*Ehd4*	-1.930	1.03E-02
Lymphocyte antigen 86	*Ly9*	-1.912	4.19E-03
Selectin, platelet (p-selectin) ligand	*Selplg*	-1.892	1.93E-03
Lysophosphatidylcholine acyltransferase 2	*Lpcat2*	-1.879	1.69E-04
Nuclear receptor subfamily 1, group D, member 2	*Nrap*	-1.858	2.67E-02
Rho GTPase activating protein 25	*Arhgap25*	-1.838	1.04E-02
Thromboxane A synthase 1, platelet	*Tbxas1*	-1.824	1.39E-03
RIKEN cDNA 5430435G22 gene	*5430435G22Rik*	-1.815	1.09E-04
Paired immunoglobin-like type 2 receptor beta 1	*Pip4k2a*	-1.813	3.88E-02
Transmembrane protein 218	*Tmem218*	-1.810	3.69E-02
Paired-Ig-like receptor A4	*Pira4*	-1.802	3.00E-04
Paired-Ig-like receptor A11	*Pira11*	-1.799	1.02E-04
Colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	*Csf2ra*	-1.793	1.54E-02
ADP-ribosylation factor 2	*Arf2*	-1.747	3.16E-02
Fermitin family homolog 3 (Drosophila)	*Fermt3*	-1.729	5.62E-03
MAP kinase-interacting serine/threonine kinase 2	*Mmp12*	-1.726	1.68E-03
C-type lectin domain family 4, member a1	*Clec4a1*	-1.717	4.20E-02
Bruton agammaglobulinemia tyrosine kinase	*Btk*	-1.706	2.16E-02
Transmembrane protein 65	*Tmem65*	-1.704	1.03E-02
Cathepsin S	*Ctss*	-1.701	1.68E-03
RIKEN cDNA 1200002N14 gene	*1200002N14Rik*	-1.689	4.40E-02
Sh3kbp1 binding protein 1	*Sh3kbp1*	-1.684	4.53E-03
Slute carrier family 16 (monocarboxylic acid transporters), member 9	*Slc16a9*	-1.680	2.43E-03
Ring finger protein 31	*Rnf31*	-1.675	7.50E-03
Prostaglandin-endoperoxide synthase 1	*Ptgs1*	-1.672	4.83E-03
Synapsin I	*Syn1*	-1.653	8.16E-04
Transcription elongation factor B (SIII), polypeptide 2	*Tceb2*	-1.639	4.38E-02
STARD3 N-terminal like	*Stard3nl*	-1.635	4.45E-03
DNA segment, Chr 12, ERATO Doi 553, expressed	*D12Ertd553e*	-1.622	1.70E-02
Filamin binding LIM protein 1	*Fblim1*	-1.612	9.65E-03
Wiskott-Aldrich syndrome homolog (human)	*Was*	-1.585	4.38E-02
Solute carrier family 8 (sodium/calcium exchanger), member 1	*Slc8a1*	-1.573	8.15E-03
Apolipoprotein B48 receptor	*Apob48r*	-1.572	4.72E-02
Eosinophil-associated, ribonuclease A family, member 10	*Ear10*	-1.570	3.82E-03
Solute carrier family 1 (glial high affinity glutamate transporter), member 3	*Slc1a3*	-1.548	4.60E-04
cDNA sequence AB124611	*AB124611*	-1.540	1.44E-02
C-type lectin domain family 1, member b	*Clec1b*	-1.535	1.77E-02
Phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	*Pira11*	-1.532	2.13E-02
Interferon-induced protein with tetratricopeptide repeats 3	*Ift20*	-1.517	2.59E-02
Selectin, lymphocyte	*Sell*	-1.493	3.43E-02
Membrane-spanning 4-domains, subfamily A, member 6D	*Ms4a8a*	-1.490	3.82E-03
Paired-Ig-like receptor A3	*Pira3*	-1.482	8.78E-04
Predicted gene, EG232801	*EG232801*	-1.472	3.82E-03
cDNA sequence BC062650	*BC062650*	-1.466	2.37E-03
Formin homology 2 domain containing 1	*Fhod1*	-1.452	2.82E-02
cDNA sequence BC065085	*BC065085*	-1.440	2.87E-02
Tumor necrosis factor receptor superfamily, member 11a	*Tnfrsf11a*	-1.433	1.06E-02
Glutamate-ammonia ligase (glutamine synthetase)	*Glul*	-1.422	2.57E-02
TMEM9 domain family, member B	*Tmem9b*	-1.407	2.05E-02
Splicing factor, arginine/serine-rich 3	*Sfrs3*	-1.407	3.24E-02
Eosinophil-associated, ribonuclease A family, member 12	*Ear12*	-1.402	5.71E-03
Legumain	*Lifr*	-1.393	2.87E-02

Lipoprotein lipase	*Lrp12*	-1.372	4.53E-02
Procollagen C-endopeptidase enhancer 2	*Pde1b*	-1.363	4.20E-02
Hairy/enhancer-of-split related with YRPW motif 1	*Hey1*	-1.357	3.22E-02
CD53 antigen	*Cd53*	-1.348	3.75E-02
Leukemia inhibitory factor receptor	*Lilrb4*	-1.343	2.73E-02
ADP-ribosylation factor-like 4C	*Arl4c*	-1.320	3.66E-02
Spindle assembly 6 homolog (C. elegans)	*Sass6*	-1.315	4.67E-03
Interleukin enhancer binding factor 3	*Ilf3*	-0.648	3.24E-02
Starch binding domain 1	*Stbd1*	0.150	4.33E-03
Folliculin	*Flcn*	0.270	3.24E-02
Adenylosuccinate synthetase like 1	*Adssl1*	0.309	4.13E-02
MAP kinase-interacting serine/threonine kinase 2	*Mknk2*	0.327	5.21E-03
Death effector domain-containing DNA binding protein 2	*Dedd2*	0.336	1.92E-02
Kruppel-like factor 13	*Klf13*	0.344	4.95E-03
NCK associated protein 1 like	*Nr1d2*	0.374	3.37E-02
Thioredoxin reductase 2	*Txnrd2*	0.385	3.78E-02
Nebulin-related anchoring protein	*Nrbp2*	0.396	4.93E-02
Aldhehyde dehydrogenase family 5, subfamily A1	*Aldh5a1*	0.401	2.82E-02
Hepatic nuclear factor 4, alpha	*Hnf4a*	0.419	4.93E-02
Solute carrier family 38, member 3	*Slc38a3*	0.433	4.24E-02
Purinergic receptor P2Y, G-protein coupled 13	*Pck1*	0.453	4.76E-02
Cobl-like 1	*Cobll1*	0.454	3.43E-02
Aminolevulinic acid synthase 2	*Alas2*	0.461	2.82E-02
Interferon regulatory factor 7	*Josd1*	0.481	4.89E-02
Acyl-Coenzyme A dehydrogenase, short chain	*Acads*	0.499	3.02E-02
Formiminotransferase cyclodeaminase	*Ftcd*	0.508	3.67E-02
CDC like kinase 4	*Clk4*	0.509	4.52E-03
Solute carrier family 2 (facilitated glucose transporter), member 9	*Slc2a9*	0.512	7.81E-03
V-raf murine sarcoma 3611 viral oncogene homolog	*Araf*	0.539	2.04E-02
Nuclear receptor binding protein 2	*Nudt19*	0.545	3.47E-02
Retinal pigment epithelium 65	*Rpe*	0.552	9.09E-03
Crystallin, zeta	*Cryz*	0.555	3.24E-02
Plastin 3	*Pls3*	0.570	3.95E-02
Pellino 2	*Per1*	0.576	1.01E-02
Interleukin 17 receptor C	*Il17rc*	0.581	4.54E-02
Phosphatidylinositol transfer protein, membrane-associated 2	*Pitpnm2*	0.590	2.73E-02
Phosphoribosyl pyrophosphate amidotransferase, transcript variant 8	*Ppat*	0.615	3.88E-02
Transmembrane protein 82	*Tmem82*	0.624	3.60E-02
Phosphoglycerate mutase 1	*Pgam1*	0.626	4.77E-02
Forkhead box O1	*Foxo1*	0.632	2.27E-02
Split hand/foot malformation (ectrodactyly) type 1	*Shf*	0.655	1.25E-02
Potassium channel tetramerisation domain containing 12b	*Klf1*	0.665	2.87E-02
Replication factor C (activator 1) 2	*Rfc2*	0.666	4.30E-02
Cyclin-dependent kinase 2	*Cdk2*	0.710	1.93E-02
Lysophosphatidylcholine acyltransferase 2	*Lphn1*	0.724	1.15E-02
Vinc finger protein 653	*Zfp653*	0.761	3.24E-02
PDZ and LIM domain 4	*Peli2*	0.768	3.22E-02
Coiled-coil domain containing 46	*Ccdc46*	0.788	4.38E-02

Female APOE*3-Leiden.CETP mice received two intraperitoneal injections of 4 ml/kg bodyweight liposomal clodronate (20 mg/kg bodyweight) at a 3-day day interval to deplete Kupffer cells from the liver, and were terminated 3 days after the second injection. RNA was isolated from liver tissue and a microarray analysis was performed. Fold change is the change in expression between 'with injection' and 'control' (no injection).

Table S3: Significantly regulated pathways.

Pathway	Source	p-value	Adjusted p-value
Classical complement pathway	BioCarta	1.43E-04	9.61E-03
Oxidative stress induced gene expression via nrf2	BioCarta	2.04E-03	3.57E-02
Fc epsilon receptor i signaling in mast cells	BioCarta	2.67E-03	4.01E-02
Chemokine signaling pathway - *Homo sapiens* (human)	KEGG	1.27E-04	9.61E-03
Prion diseases - Homo sapiens (human)	KEGG	6.61E-04	2.04E-02
Toll-like receptor signaling pathway - *Homo sapiens* (human)	KEGG	1.11E-03	2.46E-02
Osteoclast differentiation - *Homo sapiens* (human)	KEGG	1.20E-03	2.48E-02
Measles - *Homo sapiens* (human)	KEGG	1.65E-03	3.17E-02
Glycolysis / Gluconeogenesis - *Homo sapiens* (human)	KEGG	2.03E-03	3.57E-02
Lysosome - *Homo sapiens* (human)	KEGG	3.27E-03	4.76E-02
Leishmaniasis - *Homo sapiens* (human)	KEGG	3.41E-03	4.76E-02
TCR	NetPath	3.96E-05	6.22E-03
BCR	NetPath	6.27E-05	6.98E-03
EGFR1	NetPath	4.20E-04	1.77E-02
TCR signaling in naïve CD4+ T cells	PID	4.46E-04	1.77E-02
Nectin adhesion pathway	PID	3.43E-03	4.76E-02
Immune System	Reactome	2.37E-07	1.32E-04
Cell surface interactions at the vascular wall	Reactome	2.44E-05	6.22E-03
Signal regulatory protein (SIRP) family interactions	Reactome	1.04E-04	9.61E-03
Trafficking and processing of endosomal TLR	Reactome	1.92E-04	1.02E-02
Hemostasis	Reactome	2.03E-04	1.02E-02
Toll Receptor Cascades	Reactome	6.29E-04	2.04E-02
Rho GTPase cycle	Reactome	1.07E-03	2.46E-02
Signaling by Rho GTPases	Reactome	1.07E-03	2.46E-02
Adaptive Immune System	Reactome	1.20E-03	2.48E-02
Innate Immune System	Reactome	1.25E-03	2.49E-02
Astrocytic Glutamate-Glutamine Uptake And Metabolism	Reactome	2.42E-03	3.84E-02
Neurotransmitter uptake and Metabolism In Glial Cells	Reactome	2.42E-03	3.84E-02
Regulation of toll-like receptor signaling pathway	Wikipathways	4.48E-05	6.22E-03
Interferon alpha-beta signaling	Wikipathways	1.56E-04	9.61E-03
Complement Activation, Classical Pathway	Wikipathways	3.24E-04	1.50E-02
B Cell Receptor Signaling Pathway	Wikipathways	5.58E-04	2.04E-02
Signal regulatory protein (SIRP) family interactions	Wikipathways	6.41E-04	2.04E-02
Type II interferon signaling (IFNG)	Wikipathways	8.59E-04	2.46E-02
Cell surface interactions at the vascular wall	Wikipathways	9.73E-04	2.46E-02
GPCRs, Class B Secretin-like	Wikipathways	1.10E-03	2.46E-02
Toll-like receptor signaling pathway	Wikipathways	1.11E-03	2.46E-02
Interferon gamma signaling	Wikipathways	2.05E-03	3.57E-02
TCR Signaling Pathway	Wikipathways	2.51E-03	3.88E-02

Female APOE*3-Leiden.CETP mice received two intraperitoneal injections of 4 ml/kg bodyweight liposomal clodronate (20 mg/kg bodyweight) at a 3-day day interval to deplete Kupffer cells from the liver, and were terminated 3 days after the second injection. RNA was isolated from liver tissue and a microarray analysis was performed. Selected differentially expressed genes (218 genes, see Supplemental Table 2) were used as input for pathway analysis.

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