

Supplementary materials

Figure S-1. Effect of suramin on HCV genomic strand accumulation

Three days after plating, hepatocytes (FT269) were left untreated (UT) or treated with 6.2, or 62 nM r-shLDLR292, or with 2 or 8 µg/ml mAb28 against hLDLR at the time of infection. Cells were infected overnight with 25 µl of HCV(+) human serum (S241). After extensive washes the next day, cells were cultured for 5 days, the medium being renewed every 48 hours. At day 5 post-infection, cells were incubated with or without 5 mM suramin for 30 minutes to eliminate possible residual cell surface bound HCV prior to analysis. Then, cells were scraped and total RNA was extracted. One µg of RNA was analyzed for the genomic strand by real time RT-PCR. RNA quality control was evaluated by quantification of GAPDH mRNA in each sample, and amounts of HCV RNA were normalized to GAPDH mRNA; note that in this experiment the average number of HCV genome per cell is 1.5 in untreated cells.

Figure S-2. LDL binding to primary human hepatocytes

Binding isotherm of ^{125}I -LDL to primary human hepatocytes (FT257). The total binding of ^{125}I -LDL (○) was measured on duplicate wells after 90 min of incubation at 4 °C. The specific binding (●) was measured as described under "experimental procedures". The nonspecific binding (▲) represented 5 % of the total binding. Insert : The Scatchard plot of the specific binding is displayed and represents the computer-generated line of best fit, showing a single independent family of sites. The data suggest a single binding site (K_d of 7.4 nM and B_{max} values of 332 ng/mg).

Figure S-3. Effect of squalestatin and 25-hydroxycholesterol on LDL binding to primary human hepatocytes

At day 1 after plating, hepatocytes (FT236) were treated with 0.04-80 µM squalestatin (SQ) or 0.04-80 µM 25-hydroxycholesterol (25OHC) for 24 hours. At day 2, this treatment was discontinued and cells were exposed to 2.5 nM ^{125}I -LDL at 4°C (upper plot) or 24°C (lower plot) in the absence (^{125}I -LDL, control) or presence of an excess of unlabelled LDL (62.5 nM). After evaluation of LDL binding by radioactivity counting, the data were normalised first with respect to the total protein concentration and second with respect to the binding obtained in control cells taken arbitrarily at 100. These experiments are representative of other hepatocyte cultures : FT233, FT236, FT240, FT246, FT256, FT257 and FT259.

Figure S-4. Effect of squalestatin and 25-hydroxycholesterol on LDL binding to primary human hepatocytes as assessed by fluorescence analysis

At day 1 after plating, hepatocytes (FT223) were treated with 10 μ M SQ or 25OHC for 48 hours. At day 3, this treatment was continued for another 48 hours, in the presence of 3 μ g/ml BODIPY-FL LDL. Cells were fixed as indicated in Experimental, then submitted to microscope examination. NB: control, no BODIPY. These experiments are representative of other hepatocyte cultures : FT221, FT224, and FT228.

Figure S-5. Primary sequence and schematic representation of the various recombinant LDLR peptides

R1 to R7 refer to repeat motifs in the LDLR binding site. mAb12.6, mAb28 and mAb29.8 recognised respectively the following epitopes: RDCLDG, CRPD, and QDEFR.

Figure S-6. Functional characterisation of r-shLDLR peptides

A. LDL binding to LDLR soluble forms. Ninety six well plates were coated with mAb 29.8 (500ng). After washing and blocking with BSA, 50ng of indicated LDLR forms was added and incubated at 4°C overnight. Wells were blocked again and 500ng of ¹²⁵I-LDL were added in the absence or presence of a 50-fold excess of unlabeled LDL. After 1 hour at 37°C, the wells were washed, allowed to dry and the content of individual wells dissociated. After evaluation of ¹²⁵I-LDL binding by radioactivity counting, the data were normalised with respect to the binding obtained with r-shLDLR4-331 or 292 taken arbitrarily at 100.

B. Binding of lipoproteins to r-shLDLR4-331. Wells were coated with 500 ng of mAb 29.8 and 50 ng of r-shLDLR4-331; 250 to 1000 ng of ¹²⁵I-labeled LDL, VLDL or HDL were then added to the wells in the absence or presence of a 50 fold excess of the same unlabeled lipoprotein and the specific binding was determined.

C. Effect of r-shLDLR4-331 peptide on the entry of lipoproteins int CHO cells. The indicated amounts of r-shLDLR4-331 peptide were incubated for 1 hour at 37°C with 10 μ g ¹²⁵I-labeled LDL, VLDL or HDL in the absence or presence of an excess of the same unlabeled lipoprotein (40-fold). CHO cells were then exposed to this mixture for 5 hours at 37°C. After evaluation of ¹²⁵I-LDL binding by radioactivity counting, the

data were normalised first with respect to the total protein concentration and second with respect to the binding obtained in control cells (no peptide) taken arbitrarily at 100.

Figure S-7. Effect of r-shLDLR peptides on HCV RNA replicative strand accumulation

A. Three days after plating, hepatocytes (FT197) were left untreated (UT) or treated with 6.2, 62, 620 nM r-shLDLR292 (MW : 32.3 KD), r-shLDLR166 (MW : 18.2 KD) or u-hsLDLR166 at the time of infection. Cells were infected overnight with 25 μ l of HCV(+) human serum (S155). After extensive washes the next day, cells were cultured for 5 days, the medium being renewed every 48 hours. At day 5 post-infection, cells were scraped and total RNA was extracted. One μ g of total RNA sample was analysed by rTthRT-PCR for the replicative RNA strand (duplicate samples). These results are representative of observations made with other hepatocyte cultures (FT) from different donors infected with different serum samples (S) including, FT161-S42, FT167-S42, FT168-S42, FT171-S42, FT176-S42/-S12/-S31, FT195-S155, FT199-S151, FT200-S182, FT200-S183.

B. Three days after plating, hepatocytes (FT176) were exposed to HCV(+) human serum (S42). Cells were left untreated (UT) or treated with 62 nM r-shLDLR292 added either at the time of inoculation with HCV (+) serum (T0), or 30 min, 2h, or 8h later. Following overnight exposure cells were washed three times and the culture was continued. Three days post-inoculation, cells were washed three times and total cellular RNA was extracted. One μ g of total RNA was analysed by rTthRT-PCR for the replicative RNA strand (duplicate samples). These results are representative of observations made with another hepatocyte culture (FT171) infected with serum samples S12, S31, and S42.

Figure S-8. Functional characterisation of anti-LDLR monoclonal antibodies

Inhibition of LDL binding to r-shLDLR331 by monoclonal antibodies. Ninety six well plates were coated with mAb 29.8 (500ng). After washing and blocking with BSA, 50ng of r-shLDLR331 was added and incubated at 4°C overnight. Following washes and blocking, 1 μ g of indicated antibodies was added to the wells and incubation continued for 2 hours at 37°C. Then, 500ng of ¹²⁵I-LDL were added in the absence or presence of a 50-fold excess of unlabeled LDL. After 1 hour at 37°C, the wells were

washed, allowed to dry and the content of individual wells dissociated. After evaluation of ^{125}I -LDL binding by radioactivity counting, the data were normalised with respect to the binding obtained with r-shLDLR331 taken arbitrarily at 100.

Figure S-9. Effect of monoclonal antibodies against hLDLR on HCV RNA replicative strand accumulation

Three days after plating, primary hepatocytes (FT168) were exposed to 25 μl of HCV(+) serum S42, in the absence (UT) or presence of 2 or 8 $\mu\text{g/ml}$ mAbs12.6, 28 or 29.8 against hLDLR. Following overnight exposure, cells were washed three times and the culture was continued. Five days post-inoculation, cells were washed three times and total cellular RNA was extracted. One μg of total RNA sample was analysed by rTthRT-PCR for the replicative RNA strand (duplicate samples). These results are representative of observations made with other hepatocyte cultures (FT) from different donors infected with different serum samples (S) including, FT167-S42, FT197-S151, FT200-S183, FT203-S183, and FT259-S298.

Figure S-10. Effects of squalestatin, 25-hydroxycholesterol, r-shLDLR peptides and monoclonal antibodies against hLDLR on HDL entry into hepatocytes

At day 3 after plating, hepatocytes (FT263) were treated or not with 0.1, 1, 10, 20, 40, 80 μM squalestatin (SQ) or 25-hydroxycholesterol (25OHC) for 24 hours. At day 4, this treatment was discontinued and cells were exposed to 25 nM ^{125}I -HDL (2720 cpm/ng) in the absence (HDL*) or presence of an excess of unlabelled HDL (625 nM; cold). In parallel experiments, cells were cultured in the absence or presence of r-shLDLR292 or 166 (0.02, 0.2 or 2 $\mu\text{g/ml}$) or of mAbs12.6, 28 or 29.8 (2 or 8 $\mu\text{g/ml}$). After evaluation of HDL binding by radioactivity counting, the data were normalised first with respect to the total protein concentration and second with respect to the binding obtained in control cells taken arbitrarily at 100.

Figure S-11. Effect of LDL and HDL on HCV RNA replicative strand accumulation

Three days after plating, hepatocytes (FT257) were left untreated (UT) or treated with 1.5, 15 or 150nM LDL or 15, 150 or 1500nM HDL for 30 min before inoculation with 25 μl of HCV(+) serum S298. Following overnight exposure, cells were washed three times and the culture was continued. Five days post-inoculation, cells were washed

three times and total cellular RNA was extracted. One μg of total RNA was analysed by rTthRT-PCR for the replicative RNA strand. These results are representative of observations made with other hepatocyte cultures (FT) from different donors infected with different serum samples (S) including, FT197-S151 and FT259-S297.

Figure S-12. Effect of SQ on HCV RNA replicative strand accumulation

Hepatocytes (FT197) were plated and cultured under standard conditions. At day 2, cells were then treated with 0.1 to 80 μM squalestatin (SQ) or 0.1 to 40 μM 25-hydroxycholesterol (25OHC) for 24 hours. Then, cells were infected overnight with 25 μl of HCV(+) human serum (S155) at day 3. After extensive washes the next day, cells were cultured for 5 days, the medium being renewed every 48 hours. At day 5 post-infection, cells were scraped and total RNA was extracted. One μg of total RNA sample was analysed by rTthRT-PCR for the replicative RNA strand. These results are representative of observations made with other hepatocyte cultures (FT) from different donors infected with different serum samples (S) including, FT179-S155 and FT199-S151.