Interleukin 6 Stimulates Endothelial Binding and Transport of High-Density Lipoprotein Through Induction of Endothelial Lipase

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Objective—In the reverse cholesterol transport pathway, high-density lipoprotein (HDL) passes the endothelial cell barrier by mechanisms involving the scavenger receptor class B type I and the ATP-binding cassette G1. However, little is known on how inflammation influences this transendothelial transport.

Approach and Results—On stimulation with interleukin-6, cultivated primary endothelial cells showed increased binding and transport of 125I-HDL without changing the expression of scavenger receptor class B type I and ATP-binding cassette G1. Therefore, we analyzed the involvement of endothelial lipase (EL), a known HDL-binding protein expressed by endothelial cells. Here, we show an increased EL expression after interleukin-6 stimulation. Moreover, using pharmacological inhibitors or RNA interference against EL, we demonstrated its participation in HDL binding and transport through the endothelium. Furthermore, adenovirus-mediated transfection of endothelial cells with either catalytically active or nonactive EL revealed that EL facilitates the endothelial binding and transport by both bridging and lipolysis of HDL. EL was also found responsible for the reduction of HDL particle size occurring during the specific transport through a monolayer of endothelial cells. Finally, pharmacological inhibition of EL reversed the inducing effect of interleukin-6 on HDL binding and transport.

Conclusions—Interleukin-6 stimulates the translocation of HDL through the endothelium, the first step in reverse cholesterol transport pathway, by enhancing EL expression. In addition, we demonstrated the role of EL in the transendothelial transport of HDL. (Arterioscler Thromb Vasc Biol. 2013;33:2699-2706.)

Key Words: cholesterol ■ endothelial cells ■ endothelial lipase, human ■ high-density lipoprotein ■ interleukin-6 ■ inflammation

Atherosclerosis is a chronic disease characterized by lipid retention and inflammation in the arterial wall. Many studies revealed that the cholesterol concentration of plasma high-density lipoproteins (HDL) is inversely correlated with the risk of coronary artery disease events. The cardioprotective role of HDL is, in part, related to its ability to remove cholesterol from macrophage foam cells in the arterial wall and carry it to the liver for excretion into the bile. An early step in the reverse cholesterol transport is the transfer of cholesterol from macrophages to HDLs. Importantly, cholesterol efflux is not taking place in the plasma but in the arterial intima. Consequently, HDL has to cross endothelial barriers twice to get access to the cholesterol-loaded macrophages and to re-enter the blood stream for cholesterol delivery to the liver. Indeed 2 recent studies provided in vivo evidence that the transendothelial transport of HDL into the lymphatic vasculature is a rate-limiting step in reverse cholesterol transport. We previously demonstrated that endothelial cells bind, internalize, and transcytose HDL in a saturable and temperature-dependent manner. Using siRNA and pharmacological interferences, we demonstrated that endothelial cells bind and transport HDL by distinct specific mechanisms involving the scavenger receptor class B type I (SR-BI) and the ATP-binding cassette (ABC) transporter G1 but not ABCA1.

In inflammatory diseases, such as atherosclerosis, many cytokines, including tumor necrosis factor α, the interleukin (IL) 1β, and IL-6, have been demonstrated to be elevated in patient plasma. Moreover, Hingorani and Casas and Sarwar et al recently demonstrated a relationship between the IL-6 receptor pathway and atherosclerosis. IL-6 has been also demonstrated to increase the cholesterol efflux to HDL in macrophages. Consequently, we hypothesized that IL-6 may modulate the transendothelial transport of HDL. Indeed, here we provide evidences that IL-6 enhances the binding and transport of HDL in an SR-BI- and ABCG1-independent way. This observation raised the possibility that an additional protein is involved in the binding, internalization, and transport of HDL through endothelial cells. In a candidate-based
approach, we investigated whether endothelial lipase (EL) is involved. EL is a member of the triglyceride lipase family that includes hepatic lipase and lipoprotein lipase. In contrast to hepatic lipase and lipoprotein lipase, vascular endothelial cells express and secrete EL, a glycoprotein of 68 kDa. Bound by extracellular proteoglycans, EL binds HDL, hydrolyzes HDL-phospholipids at the sn-1 position, and thereby regulates plasma levels of HDL-cholesterol. By its bridging or ligand-binding function, EL, such as hepatic lipase and lipoprotein lipase, facilitates the cellular binding and uptake of different lipoproteins. Moreover, several groups demonstrated the upregulation of EL on inflammatory stimulation both in vitro and in vivo. In this study, we provide evidence that EL participates in endothelial binding, cell association, and transport of HDL.

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results
IL-6 Induces HDL Binding, Cell Association, and Transport
To investigate whether the interaction of HDL with endothelial cells is changed by the inflammatory cytokine IL-6, we stimulated aortic endothelial cells with IL-6. The interaction of HDL with the endothelial cells was characterized as binding at 4°C, cell association and transport at 37°C. IL-6 induced HDL binding to endothelial cells in a dose- and time-dependent manner (Figure IA and IB in the online-only Data Supplement). HDL binding was significantly increased after incubation with 1- or 10-ng/mL IL-6. Because of the maximal effects seen, all subsequent experiments were performed on cells that were stimulated without 0- or 10-ng/mL IL-6 for 24 hours. After 24 hours of IL-6 stimulation, specific binding was induced by 200±67% compared with not stimulated cells (100±28%; Figure 1A). Conversely, the specific cellular-associated HDL and the transported HDL were also increased by 144±21% and 178±39%, respectively, compared with not stimulated cells (100±17%; Figure 1B and 1C). Moreover, to rule out that the induction of HDL transport was because of permeability changes after IL-6 stimulation, the integrity of the endothelial cells monolayer was assessed by analyzing the permeability of H-inulin. The tracer was added to the apical chamber, and the radioactivity in the basolateral chamber was counted. The permeability coefficient was not changed after IL-6 stimulation compared with the control, namely 1.756±0.156×10–5 cm/s and 1.863±0.208×10–5 cm/s (Figure 1D) for a time period of 1 hour. In addition, to analyze whether other inflammatory cytokines stimulate HDL binding to endothelial cells, the effect of tumor necrosis factor α and IL-1β was analyzed. Both cytokines induced HDL binding after 24 hours by 130±14% and 146±33%, respectively, compared with not stimulated cells 100% ± 17% (Figure III in the online-only Data Supplement).

Previously, we demonstrated that ABCG1 and SR-BI modulate the endothelial cell interaction with HDL. Therefore, we analyzed the mRNA level and protein expression of ABCG1 and SR-BI after IL-6 stimulation. Interestingly, real-time reverse transcriptase-polymerase chain reaction analysis did not demonstrate any significant changes in the expression levels of ABCG1 and SR-BI after IL-6 stimulation, namely 1.23±0.33-fold for SR-BI and 1.10±0.49-fold for ABCG1.

Figure 1. Influence of interleukin (IL)-6 on endothelial binding at 4°C (A), cell association at 37°C (B), and transport of high-density lipoprotein (HDL; C), as well as permeability (D). Endothelial cells were stimulated with IL-6 (10 ng/mL) for 24 hours before the assays. Binding (A) was measured by incubating the cells with 10-μg/mL 125I-HDL at 4°C. To analyze the association (B) and transport (C), endothelial cells were cultivated for 24 hours on insert before IL-6 stimulation then 125I-HDL was added to the apical compartment. After 1 hour at 37°C cells were lysed, and specific activity was counted to obtain cell association (B). Medium of the basolateral compartment was harvested, and specific activity was monitored to obtain the transport (C). The permeability of the cell monolayer for insulin was determined (D). The results are represented as mean±SD of ≥3 individual experiments made of triplicate. ***P<0.001, **P<0.01. ns indicates not significant.
Relative expression compared with not stimulated cells set to 1 (Figure IIA and IIB in the online-only Data Supplement). Moreover, Western blot analyses of the protein expression levels of ABCG1 and SR-BI after stimulation were not significantly changed (1.07±0.44-fold and 0.89±0.37-fold, respectively; Figure IIC and IID in the online-only Data Supplement). These results indicated that enhanced cell binding, association, and transport of HDL after IL-6 stimulation are independent of SR-BI and ABCG1.

Identification of EL-Modulating Transendothelial Transport

We hypothesized that EL mediates HDL binding, cell association, and transport. First, we confirmed the expression of EL in endothelial cells by polymerase chain reaction (data not shown) and by Western blot analysis (Figure 2A). Then the effect of IL-6 stimulation on EL expression was analyzed. After 24 hours stimulation with IL-6, EL expression was doubled on both the RNA level (2.12±0.41-fold; Figure 2B) and the protein level (1.98±0.84-fold; Figure 2C) as revealed by real-time polymerase chain reaction and Western blotting analyses.

To evaluate the role of EL in HDL binding, we treated the cells with general pharmacological inhibitors, as well as by reducing its expression specifically through RNA interference. The binding of HDL was reduced to 79±10% after treatment of the cells with heparin. Moreover, after digesting the cell surface proteoglycans with heparinase, the binding of HDL was reduced to 65±15%. In addition, treating the cells with the general lipase inhibitor tetrahydrolipstatin reduced the binding to 53±22% compared with not stimulated cells (100±8%; Figure 3A). Finally, specific RNA interference against EL reduced binding, association, and transport to 52±12% (100±6%) for binding, 71±14% (100±5%) for association, and 47±8% (100±9%) for transport (Figure 3B–3D). On the transcriptional level, siRNA treatment led to a 90% decrease in EL mRNA expression (data not shown) and 50% decrease in EL protein level compared with noncoding siRNA transfection, whereas the expression of SR-BI and ABCG1 remained unchanged (Figure 3E). The binding of HDL to primary bovine microvascular endothelial cells and human aortic endothelial cells, as well as in the cell line EA.hy296, was reduced after tetrahydrolipstatin treatment to a similar extent as in bovine aortic endothelial cells (Figure IVA–IVC in the online-only Data Supplement). In addition, specific RNA against EL and tetrahydrolipstatin reduced HDL cell association in EA.hy926 cells (Figure IVD and IVE). These results indicate that EL modulates binding, association, and transport of HDL through endothelial cells.

Independent of the lipase activity EL, such as hepatic lipase and lipoprotein lipase, has a ligand-binding function enhancing plasma lipoprotein uptake into cells. To investigate this so-called bridging function of EL, the HDL binding and transport properties of endothelial cells overexpressing either wild-type catalytic active EL (AD-EL) or a noncatalytic mutant of EL (AD-MUT EL) were analyzed. After adenoviral infection of the endothelial cells, the protein expression of wild-type EL, as well as mutated EL, was enhanced compared with the control cells infected with AD-LacZ (Figure 4A). As expected, cells infected with AD-EL but not cells infected with AD-MUT EL showed an increased lipolytic activity (677±75% versus 92±16%) compared with the control AD-LacZ (100±24%; Figure 4B). Infecting the cells with AD-EL or AD-MUT EL increased HDL binding by 219±56% and 264±64%, respectively, compared with the control AD-LacZ–infected cells (100±19%). This indicates that the lipolytic activity of EL is not necessary for HDL binding. However, treatment with tetrahydrolipstatin significantly reduced HDL binding of endothelial cells infected with wild-type EL (AD-EL) and AD-LacZ by 58±15% and 76±6%, respectively, but not HDL binding of endothelial cells infected with lipolytic inactive EL (AD-MUT EL; Figure 4C).

EL Enhancement After IL-6 Stimulation Causes Increased HDL Binding and Transport

To analyze whether IL-6–mediated induction of EL expression, as well as HDL binding and transport, is causally related; the endothelial cells were stimulated with IL-6 and treated with tetrahydrolipstatin to inhibit EL. IL-6 stimulation enhanced binding and transport of HDL to 140±20% and 184±30%, respectively. However, tetrahydrolipstatin treatment reduced the IL-6–stimulated binding and transport by 65±17% and 77±30%, respectively, compared with IL-6 stimulation alone.

Figure 2. The role of interleukin (IL)-6 on the expression of endothelial lipase (EL). The basal expression of EL by endothelial cells was analyzed by Western blotting (A). Effects of 24-hour stimulation with IL-6 on mRNA (B) and protein levels (C) of EL were analyzed by real-time polymerase chain reaction or Western blotting. The results are represented as mean±SD of ≥3 individual experiments. *P≤0.05.
In nonstimulated cells, tetrahydrolipstatin reduced both binding and transport by 48±25% and 76±20%, respectively. The inhibitory effect of tetrahydrolipstatin on endothelial binding and transport of HDL did not differ significantly between IL-6–treated cells and untreated cells (Figure 5A and 5B). Moreover, IL-6 stimulation increased the lipase activity to 130±6% compared with the control, the IL-6 non–treated cells (Figure 5C). Transfection with specific siRNA against EL reduced the lipolytic activity by 43±30%, verifying that the lipolytic activity was exerted by EL (data not shown). Moreover, endothelial cells treated with tetrahydrolipstatin in the absence or presence of IL-6 exhibited reduced lipase activity by 58±5% and 57±7%, respectively, compared with the nonstimulated cells (100±9%; Figure 5C). These results indicate that the upregulation of EL expression is responsible for the enhancement of cell binding and transport of HDL through endothelial cells after IL-6 stimulation. Likewise, tetrahydrolipstatin reduced HDL binding to endothelial cells after tumor necrosis factor α or IL-1β stimulation by the same degree as in nonstimulated cells (Figure III in the online-only Data Supplement).

**EL Activity Is Needed for HDL Transport**

Next, we investigated the importance of the lipolytic activity of EL for HDL transport. The cells overexpressing wild-type EL (AD-EL) induced HDL transport by 244±94% compared with the control cells (AD-LacZ) 100±19% (Figure 4D). Interestingly, the cells overexpressing the mutated EL (AD-MUT EL), which showed similar HDL binding, did not increase HDL transport (98±21%) significantly beyond AD-LacZ–infected cells (100±19%; Figure 4D). To rule out that the induction of HDL transport was because of the permeability changes after EL overexpression, the integrity of the endothelial cells monolayer was assessed by analyzing the permeability of 3H-inulin after 1 hour of incubation. The permeability coefficients did not differ among cell layers infected with AD-EL (1.684±0.311×10−5 cm/s), AD-MUT EL (1.628±0.168×10−5 cm/s), and AD-LacZ (1.634±0.168×10−5 cm/s).

**EL Is Responsible for Size Reduction of HDL After Cell Contact**

We previously demonstrated that the population of HDL particle transported through a monolayer of endothelial cells is changed to a reduced size. Gel filtration analysis of the transported HDL demonstrated a Stokes diameter of 9.74±0.25 nm, whereas the Stokes diameter of the starting HDL was of 10.63±0.06 nm (Figure 6). Interestingly, the protein moiety was not changed as previously demonstrated by SDS-PAGE analysis. To assess whether EL or SR-BI is responsible for
the size reduction of HDL, we used pharmacological inhibitors, as well as RNA interference, against EL and SR-BI. After both interference with SR-BI siRNA and noncoding siRNA, transendothelial transport led to the recovery of smaller particles with Stokes diameter of 9.81±0.01 and 10.01±0.015 nm, respectively (Figure 6). In contrast, after silencing of EL, the HDL-particles that were recovered in the basolateral compartment were less prominently and not significantly reduced in size (Stokes diameter of 10.25±0.12 versus 10.63±0.05 nm for HDL without cell contact; Figure 6). To corroborate this finding, we blocked the lipase activity of EL with tetrahydrolipstatin, which prevented the size reduction of HDL (Figure 6). These data suggest that EL but not SR-BI contributes to the particle size reduction of HDL during transendothelial transport.

To investigate whether smaller HDL particles are preferentially transported, the transport of HDL₁ (1.063<d<1.125 kg/L) versus HDL₃ (1.125<d<1.21 kg/L) was analyzed. Interestingly, we could not observe any significant difference in transport through or the binding to endothelial cells (Figure V in the online-only Data Supplement).

Discussion

We previously reported that HDL is transported through a monolayer of cultivated endothelial cells in a specific process, which involves SR-BI and ABCG1. Here, we assessed the question whether HDL binding, cell association, and transport through the endothelial cells are modulated by the proinflammatory cytokine IL-6. IL-6 strongly correlates with the development of atherosclerosis, and its presence has been demonstrated in the atherosclerotic lesion.

Inflammatory cytokines have been demonstrated to modulate transendothelial transport of viruses and proteins. For example, Dohgu et al demonstrated an increase in HIV transport after IL-6 stimulation, whereas Wang et al in contrast showed a reduction of insulin transport after IL-6 stimulation. However, to our knowledge, we demonstrate for the first time that IL-6 enhances the capacity of endothelial cells to bind, to associate, and to transport HDL. Importantly, inflammatory cytokines, such as tumor necrosis factor α, IL-1β, or IL-6, have been demonstrated to increase the permeability of the endothelial barrier in a concentration and time-dependent manner. Therefore in the present study, we ruled out that the concentration of IL-6 used enhances transport by increasing the permeability of the endothelial barrier. Also unlike in human peripheral monocytes, IL-6 stimulation did not cause any changes in RNA or protein levels of SR-BI or ABCG1 in endothelial cells. These findings clearly indicate that the increased HDL binding and transport of HDL in IL-6–stimulated endothelial cells are not mediated by SR-BI or ABCG1 but by another protein. We hypothesized the involvement of EL that is produced and secreted by endothelial cells to be subsequently bound by heparan sulfate proteoglycan on the cell surface. After confirming the expression of EL in the endothelial cells, its role in HDL binding, cell association, and transport was assessed using general pharmacological treatments and specific RNA interference.

Digestion of heparan sulfate proteoglycan with heparinase to release the bound EL or competition for EL binding to heparan sulfate proteoglycan with heparin reduced HDL binding on the cell surface. Moreover, the reduction in HDL binding after blocking EL activity by the general lipase inhibitor tetrahydrolipstatin corroborates the involvement of EL in HDL binding. Interestingly, in hepatocytes, it is reported that blocking EL by tetrahydrolipstatin induces the binding of HDL. The role of EL in the binding of HDL was further confirmed by specific RNA interference against EL.
and showed a dramatic reduction in HDL binding. Specific knockdown of EL also demonstrated the involvement of EL in HDL internalization and transport of HDL through a monolayer of endothelial cells. These results clearly indicate EL as an important mediator of HDL binding in endothelial cells, as well as in hepatocytes. EL as member of the triglyceride lipase family was previously reported to bind lipoprotein via noncatalytic bridging effects.\textsuperscript{13–16} We confirmed the bridging effect of EL by demonstrating that overexpression of both catalytic active wild-type EL and inactive mutant EL increases endothelial HDL binding. These results suggest that the lipolytic activity of EL is not needed for HDL binding to endothelial cells. However, tetrahydrolipstatin treatment reduced HDL binding only in the cells overexpressing the catalytic active EL but not in the cells overexpressing the mutated EL. This suggests that in the active enzyme, tetrahydrolipstatin blocks sterically the interaction of HDL with the binding site in contrast to the mutant where the negatively charged Asp\textsubscript{192} is replaced by the uncharged Asn. Therefore, tetrahydrolipstatin is not able to bind and shield the binding site for HDL.

We previously demonstrated that the mean population size of HDL particles was reduced during transendothelial transport without degradation of the protein moiety.\textsuperscript{15} We hypothesized that either SR-BI or EL would be responsible for the effect. SR-BI is known to participate in transendothelial HDL transport. It is possible that the extraction of cholesteryl esters by the selective uptake\textsuperscript{28} reduces the particle size. Alternatively, lipolysis of phospholipids by EL\textsuperscript{29}
may also reduce the size of HDL. Therefore, we investigated whether SR-BI, EL, or both are responsible for the HDL particle size reduction in endothelial cells. Specific RNA interference experiments clearly showed that EL but not SR-BI is responsible for HDL particle size reduction. The finding was confirmed by blocking the lipolytic activity of EL with tetrahydrolipstatin.

In contrast to HDL binding the lipolytic activity of EL is essential for HDL translocation through the endothelial monolayer because only the endothelial cells overexpressing the catalytically active EL revealed an enhanced HDL transport capacity. In addition, blocking the lipolytic function of EL by tetrahydrolipstatin in primary endothelial cells reduced the HDL transport. To test whether EL enhances HDL transport by generating smaller HDL, we compared the transport rates of HDL₂ and HDL₃. The lack of any difference in the transport rates of HDL₂ and HDL₃ (Figure V in the online-only Data Supplement) rules out this explanation. An alternative explanation is that lipolytic products generated by EL from HDL that are lysophospholipids or free fatty acids modulate transendothelial transport of HDL. For example, lysophospholipids generated by EL were previously shown to modulate endothelial vasorelaxation.⁴⁰

At first sight, the induction of both EL and transendothelial HDL transport by inflammatory cytokines produces a paradox because both inflammation and the low HDL-cholesterol levels associated with high EL activity are considered as proatherogenic. However, both gene targeting in mice and Mendelian randomization experiments in humans produced controversial results on the effect of EL on atherosclerosis and cardiovascular risk, respectively.³¹–³⁵ In addition, the lipolytic products generated by EL in HDL were previously shown to exert both putatively pro- and antiatherogenic activities.³⁶–³⁹ Indeed, Hara et al³⁷ reported that EL deletion resulted in increased HDL and anti-inflammatory function in the plasma compartment; Ahmed et al³⁹ showed that HDL lipolysis by EL mediates repression of vascular cell adhesion molecule 1 expression. In view of the many anti-inflammatory properties of HDL,⁴⁰ any increased transendothelial HDL transport and hence extravascular enrichment of HDL in inflammation may be a mechanism aimed at the resolution of tissue inflammation.

In conclusion, our study demonstrated for the first time an induction of HDL binding, cell association, and transport of HDL after IL-6 stimulation. By identifying EL as the endothelial target of IL-6 stimulation, we unraveled EL as a novel contributor to transendothelial HDL transport in addition to SR-BI and ABCG1. Furthermore, our data suggest that the lipolytic products generated by EL from HDL are important for HDL transcytosis. The physiological and pathological relevance of our findings needs to be tested (eg, by animal models).

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Disclosures
None.

References
We investigated, for the first time to our knowledge, the role of inflammation on the transendothelial transport of high-density lipoprotein (HDL), which is a limiting factor in reverse cholesterol transport. We demonstrated that interleukin-6 induces HDL binding and transport through endothelial cells by upregulating endothelial lipase, which in turn binds HDL to the endothelial cell surfaces, reduces its size by lipolysis, and facilitates uptake and transcytosis. Therefore, this study unraveled endothelial lipase as a novel HDL-binding protein in endothelial cells. These results help to better understand the transport of HDL through endothelial cells. The data may also contribute to a better understanding of reverse cholesterol transport and the role of HDL and endothelial lipase in atherosclerosis.
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Material and Methods

Cell culture
Bovine aortic endothelial cells (BAECs) 1–3, bovine microvascular endothelial cells (BMEC; Cell application Inc. San Diego USA) isolated from brain vasculature and EA.hy926 (ATCC CLR-2922) were cultivated in DMEM (Sigma) supplemented with either 5% (BAECs and BMECs) or 10% FCS (EA.hy296) and human aortic endothelial cells (HAECs; LONZA Inc, Switzerland) were cultivated in EGM™-2 supplemented with SingleQuote and 10% FCS (LONZA Inc, Switzerland) at 37°C in a humidified 5% CO₂, 95% air incubator.

Endothelial binding, cell association and transport of HDL
The methods for the isolation and radiolabeling of HDL, as well as the quantification of endothelial binding, association, and transport of HDL have been described previously 1–3. In brief, plasma HDL (1.063<d<1.21 g/mL) were isolated from fresh normolipidemic human plasma of blood donors by sequential ultracentrifugation as described previously 4. Afterwards if indicated the subpopulation HDL₂ (1.063<d<1.125 kg/L) and HDL₃ (1.125<d<1.21 kg/L) were isolated by additional sequential ultracentrifugation as described previously 4. Endothelial cells were incubated with 10 µg/ml of ¹²⁵I-HDL without (total) or with a 40 fold excess of non-labeled HDL (non-specific) for 1 hour at 4 °C for the cell binding and 37 °C for the cell association and transport. Specific binding/association/transport was calculated by subtracting the non-specific binding/association/transport values from the total binding/association/transport values. All experiments were done at least three times in triplicate.

Pharmacological treatment and inhibitors
Endothelial cells were incubated with IL-6 (Preprotech, USA) at a final concentration of 10 ng/ml for 24 h in DMEM containing 0.2% BSA prior the assay. To test the involvement of EL in HDL binding, association and transport, endothelial cells were incubated with both heparin (Sigma) at a final concentration of 100 µg/ml to compete for EL binding, or with heparinase III (Sigma) at a final concentration of 0.2 U/ml to digest the glycans which bind EL for 30 minutes priors the assay. Tetrahydrolipstatine (THL) (Sigma) was added to the cells at a final concentration of 25 µM in DMEM heses containing 0.2% BSA 30 min prior adding ¹²⁵I-HDL.

siRNA transfection
Endothelial cells were transfected when 80% to 90% confluent. Sixty-seven nmol/L BLOCK-iT™ fluorescent oligo and 100 nmol/L siRNA against EL (GCA AGC AUC CUC CUU GCU UTT, GCU CGG UGA GUU CGA CAA ATT or AUC CAU GUC UUC AGC UAC ATT)
or not coding siRNA were transfected with Lipofectamine 2000 in OPTIMEM (Invitrogen) according to the manufacturer's protocol. Six hours after transfection, the medium was replaced by DMEM 5% FCS. Binding, cell association, transport and gel filtration assay were conducted between 60 and 72 hours after the transfection.

**Adenovirus generation**
Adenovirus (AD) encoding human wild type EL (AD-EL), catalytically inactive mutant EL (AD-MUT EL) containing Asp^{192} → Asn substitution and bacterial β-galactosidase (AD-LacZ) were prepared as described previously.\(^5\)

**Adenoviral infection of endothelial cells**
Endothelial cells were infected as described previously.\(^6\) In brief, confluent cell were infected with AD-EL, AD-MUT EL or AD-LacZ at MOI of 50 in DMEM without FCS. 2 hours after the infection the medium was replaced by DMEM with 5% FCS. The binding, cell association and transport were conducted 24 hours after the infection.

**Quantitative real time PCR**
Total RNA was isolated from the cells using RNA Bee (Ambio, Switzerland) according to the manufacturer's instruction. Genomic DNA was removed by digestion with DNase (Roche). Reverse transcription was performed using ReversTAD H Minus (Fermentas, Thermo Scientific) and following the standard procedure as defined by the manufacturer. Quantitative PCR was done with LightCycler FastStart DNA Master SYBR Green I (Roche). Gene specific primers were used as followed: ABCG1 (for: GAG GAA GAA AGG ATA CAA GAC C; rev: GTC AGT ATC TCC TTG ACC ATT TCC), SR-BI (for: GGA ATC CCC ATG AAC TG; rev: CTT GGG AGC TTA TGT CAT C) and EL (for: CAC CAA CAC CTT CCT GGT CT; rev: TTC TTC ATC CTC CAG CCA TC) and compared to actin (for: TGC CCT GGC ACC CAG CAC AA; rev: AGG TGG ACA GCG AGG CCA GG). Conventional PCR was carried out using following primers: GAPDH (for: CCC ATG TTC GTC ATG GGT GT; rev: TGG TCA TGA GTC CTT CCA GCA TA).

**Western Blotting**
Endothelial cells were lysed in RIPA buffer (10mmol/L Tris pH 7.4, 150 mmol/L NaCl. 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, complete EDTA (Roche)). Equal amounts of total protein were separated on a SDS-PAGE and trans blotted onto a PDVF membrane (GE Healthcare). The expression of ABCG1 (anti ABCG1 E20 Santa Cruz), SR-BI (anti SR-BI E4 Novus or anti SR-BI EP1556Y ABCAM) and EL (anti EL NB 400-118 Novus) were evaluated.
and compared to the expression of actin (anti actin AC-15, Sigma). Intensity of the blot was quantified using Image J.

**Gel filtration chromatography**

The size of HDL before and after transport studies through endothelial cells in the presence or absence of THL as well as with RNA interference against EL and SR-BI was analyzed by gel filtration chromatography as previously described. In brief, medium (0.2 ml) isolated from the basolateral compartment was loaded onto a Sephacryl S-200 HR column (60x1.6 cm) (GE-Healthcare) using an Akta-FPLC system and was eluted with Tris-buffered saline, at a flow rate of 1 ml/min. The sizes of the lipoprotein particles eluting in the various fractions were determined by comparing their $K_{av}$ values with those of marker proteins with known diameters using the LMW and HMW calibration kits (GE-Healthcare) (particle diameter range of 6.1–17 nm) as described previously.

**Lipase activity assay**

The lipase activity of endothelial cells was measured using PED-A1 (Invitrogen) following the manufacturer instruction as previously described by Darrow et al. with the following modifications. Endothelial cells (2.5x10⁶) after IL-6 or THL incubation were treated with heparinase as described previously in 0.9% NaCl. Supernatant was collected and concentrated 10 folds with 30K Amicon Ultra concentrator (Millipor) before incubating with PED-A1 for 2 hours.

**Statistical analysis**

The data for all experiment were analyzed using GraphPad Prism 5 software program. Comparisons between groups were performed using t-test and one-way ANOVA. Experiments were routinely performed in triplicate. The data have been obtained from at least 3 different experiments and are graphically represent as mean ± SD, if not indicated otherwise.

**References**


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Supplemental Figure I: Influence of IL-6 Dose- and Time-response on HDL binding.

HDL bound to endothelial cells was measured after incubation with different concentrations of IL-6 (0, 0.01, 0.1, 1 and 10 ng/ml) for 24 hours (A) or after different times (0, 6, 16, 24, 48 hours) with 10 ng/ml (B) by incubating the cells with 10 µg/ml $^{125}$I-HDL at 4 °C. The results are represented as mean ± S.D of at least 3 individual experiments made of triplicate. *** $P\leq 0.001$, ** $P\leq 0.01$, * $P\leq 0.05$ ns, not significant.
Supplemental Figure II: mRNA and protein expression of SR-BI (A, C) and ABCG1 (B, D) after IL-6 stimulation (10 ng/ml; 24 h).

The mRNA expression of SR-BI (A) or ABCG1 (B) was quantified using real time PCR and compared to the expression of actin. The protein levels of SR-BI (C) and ABCG1 (D) were evaluated by western blotting. Representative blots and quantifications of band intensities of at least 3 independent experiments are presented. The results are represented as mean ± S.D of at least 3 individual experiments. ns, not significant.
Supplemental Figure III: Influence of TNFα and IL-1β on endothelial lipase protein expression (A) and endothelial binding at 4°C (B).

Endothelial cells were stimulated with TNFα or IL-1β (both 10 ng/ml) for 24 hours prior to the assays. The Effects of TNFα or IL-1β on EL protein level (A) were analyzed by western blotting. Binding (B) was measured by incubating the cells with 10 µg/ml 125I-HDL at 4 °C in the presence or absence of a pre-incubation with 25 µM of THL. The results are represented as mean ± S.D of at least 3 individual experiments made of triplicate. **, P≤0.01, * P≤0.05, ns, not significant.
Supplemental Figure IV: Role of EL on HDL binding and cell association in BMEC, HAEC and EAhy926.

To address the role of EL in HDL binding and association to endothelial cells, bovine microvascular endothelial cells (BMEC), human aortic endothelial cells (HAEC) and EAhy926 were treated with the general lipase inhibitor THL as well as with specific specific siRNA against EL. The bound and associated HDL was assayed after 30 min pre-incubation with THL (25 µM) or 72 hours after siRNA transfection. The results are represented as mean ± S.D of at least 3 individual experiments made of triplicates. **** P≤0.0001, *** P≤0.001, * P≤0.05, ns, not significant.
Supplemental Figure V: binding and transport of HDL$_2$ and HDL$_3$.

HDL$_2$ (1.063<d<1.125 kg/L) and HDL$_3$ (1.125<d<1.21 kg/L) were isolated by gradient centrifugation and radiolabelled with iodine. Binding (A) and transport (B) were performed as described earlier in the absence or presence of 40 folds excess of non-labeled HDL (1.063<d<1.21 kg/L). The results are represented as mean ± S.D of at least 3 individual experiments made in triplicate.