Long Pentraxin 3, a Key Component of Innate Immunity, Is Modulated by High-Density Lipoproteins in Endothelial Cells

Giuseppe Danilo Norata, Patrizia Marchesi, Angela Pirillo, Patrizia Uboldi, Giulia Chiesa, Virginia Maina, Cecilia Garlanda, Alberto Mantovani, Alberico Luigi Catapano

Objective—High-density lipoproteins (HDL) are endowed with cardiovascular protective activities. In addition to their role in reverse cholesterol transport, HDL exert several beneficial effects on endothelial cells, including the induction of endothelial nitric oxide synthase and prostacyclin release, and the control of the immune and inflammatory response.

Methods and Results—To identify possible mechanisms involved in these effects we investigated the modulation of the expression of acute phase proteins of the pentraxin superfamily, such as C-reactive protein (CRP), serum amyloid P component (SAP), and the long pentraxin 3 (PTX3) by HDL in human endothelial cells. HDL induced PTX3 mRNA expression and protein release, whereas no effect was observed on CRP and SAP expression. This effect was mainly dependent on the activation of the lysosphingolipids receptors-PI3K/Akt axis and was mimicked by sphingosine 1 phosphate and other S1P mimetics. This observation was confirmed in vivo; indeed an increased expression of PTX3 mRNA was detected in the aorta of transgenic mice overexpressing human apoA-I, compared to apoA-I knock-out mice. Furthermore, plasma levels of PTX3 significantly increased in C57BL/6 mice injected with HDL.

Conclusions—These data suggest that part of the atheroprotective effects of HDL could result from the modulation of molecules that act as sensors of the immunoinflammatory balance in the vascular wall. (Arterioscler Thromb Vasc Biol. 2008;28:925-931)

Key Words: PTX3 ■ HDL ■ endothelium ■ S1P

Innate and adaptive immune responses participate in several phases of atherosclerosis.1 The process of plaque formation is initiated by biologically active lipids, such as those found in oxidized low-density lipoproteins (ox-LDL), generated in the innermost layer of the artery, which activate a cascade of immune cell recruitment and activation in the atherosclerotic plaque. Monocyte-derived macrophages abound in plaque, where they are recruited by locally produced chemokines. Macrophages uptake lipids through the scavenger receptors, become foam cells, and promote MHC-class II antigen presentation of internalized material to CD4+ T cells, thereby linking innate and adaptive immunity.1 However, mice lacking scavenger receptors showed increased atherogenesis,2 thus suggesting that receptor-mediated internalization of modified lipoproteins by macrophages can facilitate the elimination of these particles through high density lipoprotein (HDL)-dependent mechanisms.1

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HDL, in addition to their role in reverse cholesterol transport, influence different functions of endothelial cells,3–5 including angiogenesis, vasorelaxation, and cell proliferation, via the modulation of the expression and of the activity of different genes, such as endothelial nitric oxide synthase (eNOS),5 cyclooxygenase (COX), prostacyclin synthase (PGI-S),3,4 adhesion molecules,6 and proteases as ADAMTS-1.7 Of note, the expression of several genes involved in the immune response is related to HDL plasma levels. For instance, the levels of the antiinflammatory cytokine transforming growth factor beta (TGF-β)1 are modulated by HDL in endothelial cells and in the arterial wall,8 raising the possibility that HDL could affect vascular immune and inflammatory responses.

The long pentraxin 3 (PTX3) is a member of the pentraxin superfamily, a family of acute phase proteins highly conserved during evolution and characterized by a multimeric, usually pentameric, structure.9 PTX3 shares similarities with the classical short pentraxins such as C-reactive protein (CRP) and serum amyloid P component (SAP); however, it has an unrelated long N-terminal domain coupled to the C-terminal pentraxin domain and differs in gene organization, cellular source, and ligands recognized.10 PTX3 is produced...
rapidly and released by several cell types in response to primary inflammatory signals, in particular by mononuclear phagocytes, dendritic cells (DC), fibroblasts, and endothelial cells. PTX3 modulates the classical pathway of complement activation and facilitates pathogen recognition by macrophages and DC. Recent studies in gene-modified mice have shown that PTX3 plays complex, nonredundant functions in vivo, ranging from the assembly of a hyaluronic acid-rich extracellular matrix and female fertility to innate immunity against diverse microorganisms. In this article we investigated the effect of HDL on the expression of genes belonging to the pentraxin family, and we report that HDL specifically induce PTX3 expression via activation of the PI3K/Akt pathway, whereas has no effect on CRP and SAP expression. This finding was confirmed in vivo in C57BL/6 mice after injection of HDL and in transgenic mice overexpressing human apoA-I, which compared with apoA-I knockout mice, showed increased expression of PTX3 mRNA in the aorta.

**Materials and Methods**

**Materials** HDL subfraction 2 (1.063 to 1.125 g/mL) and 3 (1.125 to 1.21 g/mL) were obtained from freshly isolated human plasma by preparative ultracentrifugation and dialyzed versus PBS containing 0.01% EDTA. HDL were used within 6 h of preparation, and the concentration is expressed as protein. Human apolipoprotein A-I (apo A-I) was purified as described. Discoidal reconstituted-HDL containing palmitoyloleoyphosphatidylcholine (POPC; Sigma) and apo A-I (r-HDL) (POPC/apo A-I molar ratio 100:1) or POPC, sphingosine 1-phosphate (SIP; Sigma), and apo A-I (r-HDL/SIP) (POPC/SIP/apo A-I molar ratio 89:11:1) were prepared by the cholate dialysis method.

Human umbilical vein endothelial cells (HUVECs) were isolated and cultured as previously described. Human aortic endothelial cells (HAECs) (Cambrex, Italy) were cultured under the same conditions of HUVECs and were used between 3rd and 5th passage. In all experiments, cells were preincubated with serum-free medium for 6 h and incubated with HDL, for 4 h. Control cells were incubated for 4 h with the experimental medium containing the same percentage of PBS that was added with the stimulus.

SIP was used at 10 μmol/L; Apo A-I (Sigma) was used at 100 μg/mL; FTY-720 (Alexis), FTY-720-phosphorylated and VPC24191 (both kind gift of Dr Nofer, University of Munster, Germany) were used at 10 μmol/L, and SEW2871 (Sigma) was used at 20 nmol/L. The MEK inhibitor U 0126 (New England Biolabs), the PI3-K inhibitor Ly 294002, and the Akt inhibitor SH-5 (both from Alexis) were used at a final concentration of 10 μmol/L, 50 μmol/L, and 10 μmol/L, respectively. Suramin (Calbiochem) was used at a final concentration of 0.1 mmol/L. At these concentrations the inhibitors effectively decreased the phosphorylation of the downstream targets (supplemental Figure I, available online at http://atvb.ahajournals.org).

**Quantitative Real-Time Polymerase Chain Reaction**

Total RNA was extracted and reverse transcribed as described. Three μL of cDNA were amplified by real-time quantitative polymerase chain reaction (PCR) with 1X Syber green universal PCR mastermix (BioRad). The specificity of the syber green fluorescence was tested as described. The primers used are indicated in supplemental Table I. Each sample was analyzed in duplicate using the IQ-Cycler (BioRad). The PCR amplification was related to a standard curve ranging from 10^-15 mol/L to 10^-11 mol/L.

**PTX3 Protein Expression**

Human PTX3 was measured with a sandwich ELISA as described. Murine PTX3 was measured with a sandwich ELISA using 2 mAb antiserum pt3 (2C3 and 6B11). The ELISA assay did not cross-react with the short pentraxins CRP and SAP.

**Animal Studies**

The generation of transgenic mice expressing human apo A-I and deficient in the endogenous murine apo A-I (hA-I) has been described previously. Ha-I transgenic mice and murine A-I knock-out mice (A-I^-/-; 10 males and 10 females in each group) were fed a standard diet for 18 wk and euthanized by exposure to carbon dioxide according to the protocol of the internal ethical committee. After perfusion with saline, the aortas were isolated, placed in a storing solution (RNA later, Ambion) at -20°C, or paraffin embedded. For RNA isolation the samples were homogenized in a dismembrator (B Braun, Melsungen AG, Germany), then processed as described.

HDL (10 mg apoA-I/kg of body weight was administered intravenously via the tail veins to 14- to 18-wk-old C57BL/6J mice (n=10; 5 male and 5 females). PBS and lipopolysaccharide (LPS) (1 μg/Kg) injected animals (n=10; 5 male and 5 females) from the same batch served as negative and positive controls, respectively. 18 h after the injection animals were processed as mentioned above.

**Plasma Lipid Analysis**

Blood samples were collected in EDTA tubes immediately before death by retro-orbital bleeding and plasma was separated by low-speed centrifugation at 4°C. The measurement of plasma lipids was performed by standard enzymatic techniques (ABX for Cobas Mira Plus); HDL-C was determined after precipitation of apoB-containing lipoproteins.

**Statistical Analysis**

For in vitro studies data are means±SD and are based on 5 separate experiments. Statistical analysis was performed by unpaired Student test with the use of the SPSS 12.0 for Windows.

**Results**

**HDL Induces PTX3 Expression in Human Endothelial Cells**

First we investigated the effects of HDL on the mRNA expression of PTX3, CRP, and SAP by real-time PCR. The HDL3 effect on PTX3 expression in HUVECs was dose dependent (Figure 1A) in a range from 10 μg/mL to 500 μg/mL of protein. The maximal effect on PTX3 mRNA expression was observed at 4 h (Figure 1B). These findings were confirmed at the protein level. PTX3 protein release was significantly increased in a time- and dose-dependent fashion (Figure 1C and 1D). The threshold for SAP and CRP was observed after 40 cycles of amplification, suggesting extremely low expression of these genes in the endothelium under our experimental conditions.

The effect of HDL on PTX3 induction was confirmed in a second endothelial cell type, namely HAECs. Incubation with HDL3 (200 μg/mL) induced both PTX3 mRNA expression and protein release (2.47±0.83-fold and 2.46±0.81-fold, respectively; P<0.05 for both; Figure 2A and 2B). This effect was specific for the HDL class, indeed also HDL2-induced PTX3 expression, although to a lower extent compared to HDL3 whereas no induction was observed in the presence of LDL or modified LDL (supplemental Figure II).

Next we investigated whether this effect was specific for endothelium or was common for other cell types know to
express PTX3, such as monocytes. U937 cells were incubated with HDL3 (200 μg/mL) for 4 h and PTX3 mRNA expression was investigated. No effects on PTX3 expression was observed (data not shown).

HDL have been shown to activate several intracellular pathways in endothelial cells, including the ERK1/2 pathway and the PI3K/Akt pathway. In HUVECs, preincubation for 1 h with LY 294002 or SH-5 completely abolished PTX3 mRNA induction by HDL3 (from 2.78 ± 0.68 to 0.95 ± 0.54, 0.91 ± 0.37-fold for LY294002 and SH-5 respectively, P < 0.01 for both), whereas no effect on preincubation with U0126 was observed (1.98 ± 0.34, P = ns; Figure 2C). When the effect of intracellular kinase inhibitors on HDL-induced PTX3 protein release was assessed, only the inhibition of PI3K/Akt axis affected HDL-mediated induction, whereas no difference was observed in the presence of the ERK1/2 inhibitor (Figure 2D).

Next we investigated the nature of the stimulatory component present in HDL responsible for PTX3 mRNA induction. First HUVECs were incubated with HDL in the presence or absence of pertussis toxin and suramin (an antagonist of lysosphingolipid receptor EDG-322; Figure 3A). Both pertussis toxin and suramin inhibited HDL-mediated PTX3 expression, suggesting the possible involvement of a lysosphingo-
lipid molecule in HDL mediated effects. To confirm the involvement of an S1P receptor, siRNA experiments were performed. Under conditions in which siRNAs specifically inhibited the respective S1P receptor or SR-B1 expression (Figure 3B), HDL-mediated PTX3 mRNA induction was decreased in cells treated with siRNA against S1P1 or S1P3 whereas no differences were observed in cells treated with siRNA against SR-B1 (Figure 3C).

To confirm the involvement of a lysosphingolipid-dependent pathway, reconstituted HDL were prepared either containing ApoA-I/POPC (r-HDL) or ApoA-I/POPC/S1P (r-HDL/S1P). When the effect on PTX3 expression was investigated only r-HDL/S1P (100 µg/mL of protein) induced PTX3 expression (Figure 4A). Therefore, HUVECs were incubated with S1P, Apo A-I, or S1P mimetics such as FTY-720, FTY-720 phosphate (FTY-720P), VPC 24191, and a S1P1 receptor agonist SEW2871. S1P induced PTX3 mRNA expression to an extent comparable to that of HDL (2.86 ± 0.91-fold; *p<0.05), whereas Apo-AI did not affect PTX3 expression (Figure 4B). The effect of S1P was inhibited by suramin (an antagonist of lysosphingolipid receptor EDG-322; Figure 4B); also LY294002 inhibited the SIP-mediated PTX3 induction (Figure 4B). Finally, when cells were incubated with S1P mimetics, a significant induction of PTX3 was observed (Figure 4B), confirming a major role of the S1P_EDG1/3 pathway in determining the observed effect.

Increased HDL Levels in Animal Models Are Associated With Increased PTX3 Expression in the Vascular Wall

To confirm ex vivo the role of HDL on PTX3 expression, we analyzed PTX3 levels in the aortas and plasma of hA-I mice compared to those of A-I/ /mice. These 2 mouse models with identical genetic background were chosen with the aim of comparing an in vivo model of low HDL with one of high HDL levels (HDL-cholesterol levels at sacrifice 11.9 ± 4.9 mg/dL and 124.2 ± 31.1 mg/dL, respectively). In addition we investigated PTX3 levels also in C57Bl6 mice. PTX3 expression was significantly increased in the aortas of transgenic hA-I and C57Bl6 mice compared to A-I/ /mice (P<0.01 for both; Figure 5A). Blaschke et al recently showed that also CRP and SAP mRNA expression can be modulated in mice23; we therefore investigated also their expression in...
the aorta, but no differences were observed among the 3 mice models (Figure 5A). When PTX3 protein levels were measured in aortas and plasma, no difference was observed in plasma levels between the 3 groups (Figure 5C), whereas significantly increased levels were observed in the aorta (Figure 5B), possibly suggesting that the effect of HDL is mainly localized to the arterial wall.

To further address this issue we injected C57BL/6 mice with plasma-derived HDL (500 μg ApoA-I per mouse) and compared the effect with that of LPS (10 μg/Kg). HDL treatment significantly increased PTX3 mRNA and protein levels in the aortas of mice (P<0.05; Figure 6A and 6B), similar differences were observed in the presence of LPS. On the contrary, PTX3 plasma levels were significantly increased only on LPS injection (372±193 ng/mL versus 15.4±8.1 ng/mL; P<0.05), whereas a nonsignificant increase was observed on HDL injection (22.4±7.2 ng/mL; Figure 6C).

Finally we tested the effects of a S1P mimetic (FTY-720) on PTX3 expression in Apo A-I KO mice. Injection of FTY-720 (20 μg per mouse) resulted in an increase of PTX3 mRNA levels (2.6±0.7-fold), and aorta protein levels compared to PBS injection (from 1.67±0.47 ng/mL to 2.75±0.33 ng/mL; P<0.05 for both). No significant differences were observed on PTX3 plasma levels between PBS and FTY injection (18.9±3.9 ng/mL versus 24.5±17.3 ng/mL; P=ns).

**Discussion**

Our study shows that HDL induce the expression of PTX3 both in vitro and in vivo. This effect is dependent on the activation by lysosphingolipids of a G-coupled receptor belonging to S1P-receptors family and the subsequent activation of the PI3K/Akt pathway. Our data add new insights suggesting a further mechanism by which HDL may play an atheroprotective effect through the modulation of a key component of the humoral arm of innate immunity.

PTX3 has been initially described as an early marker for primary local activation of innate immunity and inflammatory response and is highly expressed in the heart during inflammatory reactions, in atherosclerotic lesions, in plasma in patients with arterial inflammation, particularly with un-

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**Figure 5.** PTX3 mRNA and protein expression in the aortas of human apoA-I transgenic mice (hA-I) and C57Bl6 mice compared to apoA-I knockout mice (A-I). A, PTX3, CRP, and SAP mRNA expression in the arterial wall. B and C, PTX3 protein levels in arterial wall and plasma. (For detailed figure legend please see http://atvb.ahajournals.org).

**Figure 6.** Effects of intravenously injection of HDL or LPS on arterial wall and plasma PTX3 levels. HDL, LPS, or saline were administered intravenously via a tail vein. A, PTX3 mRNA levels in the arterial wall. B and C, PTX3 protein levels in the arterial wall and in plasma respectively. (For detailed figure legend please see http://atvb.ahajournals.org).
stable angina pectoris, and in vascular cells in response to inflammatory stimuli and to oxLDL.\textsuperscript{27–30}

Whether PTX3 plays a role in the pathogenesis of vascular diseases, however, is unclear. The in vivo role of PTX3 in inflammatory conditions has been investigated using PTX3 overexpressing and deficient mice.\textsuperscript{24} PTX3 is a potent inhibitor of the autocrine and paracrine stimulation exerted by fibroblast growth factor-2 on smooth muscle cells in vitro, and PTX3 overexpression in transduced SMCs reduces intimal hyperplasia after arterial injury as a result of direct binding to FGF2.\textsuperscript{31} In a model of cecal ligation and puncture, PTX3 overexpression resulted in increased resistance to LPS toxicity.\textsuperscript{10} Furthermore, in a model of AMI caused by coronary artery ligation, PTX3-deficient mice show exacerbated heart damage associated with a greater no-reflow area and increased inflammatory response; a phenotype which is reversed by exogenous PTX3 (Savio et al, unpublished). These data support a cardioprotective function of PTX3 and are in line with the observation that its expression could be induced by HDL.

In endothelial cells the effect of HDL\textsubscript{3} is specific for PTX3, as the expression of 2 others pentraxin such as CRP and SAP is not affected, and is dependent mainly on PI3K/Akt activation. This observation is in agreement with the known role of Akt pathway in mediating HDL-induced endothelial gene expression\textsuperscript{3,8} and support a protective role for HDL on the endothelium.\textsuperscript{3,4}

A lysosphingolipid, S1P, carried by HDL and responsible for HDL induced NO-dependent vasorelaxation,\textsuperscript{32} antiapoptotic effects,\textsuperscript{33} and TGF-β2 induction,\textsuperscript{8} was responsible for PTX3 induction. The involvement of the lysosphingolipids/lysosphingolipids receptors pathway in this response was also confirmed first by the observation that silencing of the lysosphingolipids receptor S1P1 and S1P3 inhibited the effect of HDL on PTX3 expression and second by the observation that several S1P mimetics induced PTX3 expression. These findings provide insights into the HDL components responsible for this effect. Accordingly, apo A-I, lipid free or incorporated in r-HDL, failed to induce PTX3 expression in endothelial cells, whereas in vivo injection of HDL induced PTX3 expression. This is not the first observation of a protective signal inducing PTX3 expression. Indeed also interleukin (IL)-10, a cytokine with atheroprotective properties,\textsuperscript{34} enhances PTX3 production,\textsuperscript{35} suggesting that PTX3, as a key component of the innate immunity, is regulated by both proinflammatory stimuli, such as LPS, tumor necrosis factor (TNF) alpha, and IL1β, and antiinflammatory molecules, such as IL-10 and HDL.

The endothelium integrates the signals generated by multiple factors and regulates the immuno-inflammatory response. This is achieved by the activation of antiinflammatory counter-regulatory mechanisms that finely tune vascular inflammation and maintain the integrity and homeostasis of the vascular wall.\textsuperscript{10} In this view, it is possible that PTX3 acts as a molecule at the crossway between proinflammatory and antiinflammatory stimuli perhaps counterbalancing the over-activation of a proinflammatory proatherogenic cascade. This observation is substantiated by the observations that PTX3-deficient mice display an higher degree of ischemic injury in the heart\textsuperscript{24} and overexpression of PTX3 reduces intimal thickening after balloon injury.\textsuperscript{31} Other molecules at the crossroad between proinflammatory and antiinflammatory responses, such as TGF beta or Cox-2,\textsuperscript{37} are modulated by HDL in the endothelium.\textsuperscript{4,8} This suggests that part of the atheroprotective effects of HDL could result from the modulation of molecules that act as sensors of the immuno-inflammatory balance in the vascular wall.

In summary, HDL induces PTX3 expression in vitro and in vivo, mainly via PI3K/Akt activation. Because PTX3, beyond its role as a multifunctional soluble pattern recognition receptor, acts as a nonredundant component of the humoral arm of innate immunity by tuning inflammation and modulating matrix deposition,\textsuperscript{24} we believe that our findings help in identifying a novel mechanism by which HDL may exert their protective effects on endothelial cells and vascular wall function.

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**Disclosures**

None.

**References**


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Figure I

Effectiveness of kinases inhibitors in decreasing the phosphorylation of the downstream targets

In particular in panel A, the effect of SH5 in inhibiting AKT phosphorylation and also the phosphorylation of a Akt downstream effector (GSK) are shown. In panel B the effects of U0196 (MEK1/2 inhibitor), LY294002 (PI3K inhibitor), SH5 (Akt inhibitor) and SB203580 (P38MAPK inhibitor) on HDL3-induced phosphorylation of ERK1/2, P38MAPK and CREB are shown.
### Table I

<table>
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<tr>
<th>Gene</th>
<th>Primer forward</th>
<th>Primer reverse</th>
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<td>hPTX3</td>
<td>GGGACAAGCTCTTCATCATGCT</td>
<td>GGGACAAGCTCTTCATCATGCT</td>
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<td>hCRP</td>
<td>GCGGGGCCCCTCAGCTCTTA</td>
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<td>mRLP13a</td>
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h, human; m, mouse.
Specific modulation of PTX3 expression by HDL.

When endothelial cells were incubated with different lipoproteins for 4 hours, the induction of PTX3 was specific for the HDL class, indeed also HDL2 (200µg/mL) induced PTX3 expression although to a lower extent compared to HDL3 while no induction was observed in the presence of LDL (100µg/mL) or copper oxidized LDL (Ox-LDL) or lypoxigenase modified LDL (LoxLDL) (both at 50µg/mL).
Detailed figure legends

Figure 1

**Effects of HDL on PTX3 expression in HUVECs.** Panel A: Concentration dependent effect of HDL₃ on PTX3 mRNA expression. Panel B: Time dependency of the effect of HDL₃ on PTX3 mRNA expression. Panel C: Concentration dependent effect of HDL₃ on PTX3 protein expression. Panel D: Time dependency of the effect of HDL₃ on PTX3 protein expression. In all panels quantitative real time PCR results or ELISA results are shown (mean ±SD from 4 separate experiments, each performed in duplicate).

Data are normalized for RLP13a expression. For experimental details see material and methods section. (*p <0.05 and ** p<0.01 vs control cells)

Figure 2

**Effects of HDL on PTX3 expression in HAECs and Intracellular signalling pathways involved in HDL-dependent PTX3 induction.** Panel A: effect of HDL₃ (200µg/mL) on PTX3 mRNA expression. Panel B: effect of HDL₃ (200µg/mL) on PTX3 protein expression. Panel C: Effect of ERK 1/2 (U), PI3K (LY) or Akt (SH5) inhibition on HDL(200µg/mL)-induced PTX3 expression. Panel C. Effect of ERK 1/2 (U), PI3K (LY), and Akt (SH5) inhibition on HDL(200µg/mL)-induced PTX3 protein release. Quantitative real time PCR results (panel A and C) or ELISA results (panel B and D) are shown (mean ±SD from 4 separate experiments, each performed in duplicate). For Q-PCR data are normalized for RLP13a expression. For experimental details see material and methods section (*p<0.05 vs ctrl; §p <0.01 vs HDL).

Figure 3

**Involvement of lysosphingolipid receptors in HDL-mediated PTX3 mRNA induction.** Panel A. Effect of pertussis toxin and suramin on HDL mediated PTX3 mRNA expression (*p<0.05 vs ctrl;
§p <0.01 vs HDL). Panel B. mRNA expression of S1P1, S1P3 and SR-B1 in endothelial cells treated with siRNA against S1P1, S1P3 and SR-B1 or non silencing siRNA (control) (* p<0.01 vs siRNA scramble). Panel C. Effect of silencing of S1P1, S1P3 or SR-B1 on HDL-mediated PTX3 mRNA expression (* p<0.01 vs ctrl; § vs siRNA scramble). Quantitative real time PCR results (mean ±SD from 4 separate experiments, each performed in duplicate) are shown. In all panels data are normalized for RLP13a expression. For experimental details see material and methods section.

Figure 4

Effects of sphingosine 1 phosphate and S1P mimetics on PTX3 mRNA expression.

Panel A. Effect of reconstituted HDL, containing ApoA-I/POPC (r-HDL) or ApoA-I/POPC/S1P (r-HDL/S1P) (both 100µg/ml of protein) on PTX3 mRNA expression (*p<0.05 vs ctrl). Panel B. Effect of S1P, lipid free Apo A-I, FTY-720, FTY-720 phosphate (FTY-720P), VPC24191 and SEW2871 and role of suramin and PI3K inhibition (LY) of S1P signalling on PTX3 mRNA expression (*p<0.05 vs ctrl; §p <0.01 vs S1P). Quantitative real time PCR results (mean ±SD from 4 separate experiments, each performed in duplicate) are shown. In all panels data are normalized for RLP13a expression. For experimental details see material and methods section.

Figure 5

Expression of PTX3, CRP and SAP in the aortas of human apoA-I transgenic mice (hA-I) and C57Bl6 mice compared to apoA-I knockout mice (A-I -/-). Panel A. Quantitative real time PCR results (mean ± SD of mRNA from 20 animal for each group) are shown (*p <0.05 vs Apo A-I -/- mice). Data are normalized for RLP13a expression. Panel B and C. Arterial wall and plasma PTX3 protein levels are shown (*p <0.05 vs Apo A-I -/- mice). For experimental details see material and methods section.
Figure 6

Effects of intravenously injection of HDL or LPS on arterial wall and plasma PTX3 levels.

HDL, LPS or saline were administered intravenously via a tail vein. Animals were euthanized 18 hours after receiving the injections, plasma was collected and the aortae were removed for quantification of PTX3 mRNA levels by Q-RT-PCR or protein levels by ELISA. PTX3 mRNA levels in the arterial wall are shown in panel A, PTX3 protein levels in the arterial wall are shown in panel B, while PTX3 plasma levels are shown in panel C (*p <0.01 vs PBS).