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Lipopolysaccharide downregulates CD91/low-density lipoprotein receptor-related protein 1 expression through SREBP-1 overexpression in human macrophages

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ABSTRACT

Sterol regulatory element-binding proteins (SREBPs) negatively modulate the expression of the CD91/low-density lipoprotein receptor-related protein (LRP1), a carrier and signaling receptor that mediates the endocytosis of more than 40 structurally and functionally distinct ligands. The aim of this work was to analyze whether lipopolysaccharide (LPS) can regulate LRP1 expression through SREBPs in human monocyte-derived macrophages (HMDM). LPS led to LRP1 mRNA and protein inhibition in a dose- and time-dependent manner. Concomitantly, a strong upregulation of SREBP-1 mRNA and SREBP-1 nuclear protein levels was observed in LPS-treated HMDM. The specific silencing of SREBP-1 efficiently prevented LRP1 reduction caused by LPS. SREBP-1 mRNA and nuclear protein levels remained high in HMDM treated with LPS unexposed or exposed to LDL. Native (nLDL) or aggregated LDL (agLDL) *per se* downregulated SREBP-2 expression levels and increased LRP1 expression. However, lipoproteins did not significantly alter the effect of LPS on SREBP-1 and LRP1 expression. Collectively, these data support that lipoproteins and LPS exert their modulatory effect on LRP1 expression through different SREBP isoforms, SREBP-2 and SREBP-1, respectively. These results highlight a crucial role of SREBP-1 as a mediator of the downregulatory effects of LPS on LRP1 expression in human macrophages, independently of the absence or presence of modified lipoproteins.

1. Introduction

Inflammation is a beneficial response triggered by infection or tissue injury. Deregulated or excessive inflammation, however, may contribute to disease pathogenesis in cardiovascular and autoimmune diseases, cancer and asthma. Identifying pro- and anti-inflammatory factors and pathways is a major challenge in these diseases.

CD91/low-density lipoprotein receptor-related protein 1 (LRP1) is a member of the LDLR gene family. It mediates the endocytosis of more than 40 structurally and functionally distinct ligands, including proteases and growth factors implicated in inflammation [1,2]. LRP1 also regulates the cell surface abundance of other membrane proteins, some of which have cell-signaling activity [3,4]. Recent studies suggest that LRP1 may regulate cell survival [5], angiogenesis [6,7], and inflammation [8,9]. Overton et al. proposed that the activity of LRP1 in atherosclerosis may be linked to its ability to suppress local inflammation [10]. Inflammation is a key process in the onset and progression of atherosclerosis, and leukocytes and proinflammatory cytokines are present in all the stages of progression of atherosclerotic lesions [11,12]. The risk of atherosclerosis is increased in obese subjects and type 2 diabetes patients as they have a chronic low-grade inflammation [13]. Plasma from these patients contains low doses of lipopolysaccharide (LPS) [13,14]. LPS is a strong risk factor for atherogenesis [15] and infection with gram-negative bacteria increases the morbidity and mortality of atherosclerosis-related cardiovascular diseases [16,17]. Gram negative bacteria are associated with endotoxins, and macrophages react to endotoxins by modulating the expression of transcription factors involved in lipid and cholesterol synthesis, such as sterol regulatory element-binding proteins (SREBPs) [18]. SREBP-1a, the most abundant isoform in macrophages, acts as a link between lipid metabolism and the innate immune response

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[19,20]. Our group has previously reported that SREBP-1 and SREBP-2 play a repressor role in the expression of low-density lipoprotein receptor-related protein 1 (LRP1) transcription [21]. Macrophage LRP1 modulates inflammation in atherosclerotic plaques through several processes, such as ingestion of the apoptotic cells/debris and macropinocytosis [22,23] and regulation of extracellular cytokine levels [10]. Macrophage LRP1 has been reported to participate in lipid-loaded macrophage foam cell formation [24–26]. LRP1 interacts with aggregated (agLDL). The physical characteristics of agLDL seem to determine a particular uptake mechanism. Macrophages can hydrolyze extracellularly agLDL-cholesteryl esters (CE) by creating extracellular acidic compartments where lysosomes deliver acid hydrolases [27]. As a result, local membrane free cholesterol increases causing local actin polymerization and membrane extension, and promoting contact between plasma membrane and agLDL. Both agLDL-induced membrane extension and agLDL-induced LRP1 overexpression [21,28] may contribute to the strong capacity of this modified lipoprotein to induce macrophage foam cell formation. Interestingly, membrane extension also underlies the primary response of macrophage to microbial toxins [29]. In this process, new lipids come mainly from endoplasmic reticulum (ER) [30], where SREBPs are located.

We hypothesize that the mechanisms underlying the interplay between SREBPs and LRP1 may be key to understanding the complex crosstalk between lipids and inflammation. The aims of this work were to analyze the effect of LPS on LRP1 expression and to determine the role of SREBP-1 and SREBP-2 in this effect in human macrophages. We also analyzed LRP1 macrophage regulation by LPS in absence or presence of aggregated LDL and evaluated the functional capacity of LRP1 to take up agLDL in LPS-stimulated macrophages.

2. Material and methods

2.1. Reagents

RPMI medium, penicillin/streptomycin and HEPES were from Gibco (Carlsbad, CA), and human AB serum was purchased from Lonza (Walkersville, MD). LRP1, SREBP-1 and SREBP-2 specific siRNAs were synthesized by Applied Biosystems (Foster City, USA). The Silencer™ siRNA Transfection kit was obtained from Ambion, Life Technologies (Grand Island, NY 14072). Tripure™ isolation reagent was from Roche Molecular Biochemicals. Antibodies anti-LRP1 β chain were purchased from R&D (Minneapolis, MN 55413), anti-β-actin from Sigma (St. Louis, MO), anti-SREBP-1 from Santa Cruz Biotechnology, Inc (2145 Delaware Avenue, USA) and anti-SREBP-2 from MBL (Amicon-Millipore, Billerica, MA). SREBP-2 antibodies for immunohistochemistry were purchased by Abcam (Cambridge, MA 02139-1517, USA). Enhanced chemiluminescence reagents were from Pierce (Rockford, IL). Endotoxin levels of lipoproteins were analyzed using the Limulus amebocyte lysate test obtained from Bio Whittaker (Lonza, Walkersville, MD). Organic solvents for lipid extraction were obtained from Sigma (St. Louis, MO) and thin layer chromatography plaques from Macherey–Nagel GmbH & Co. KG.

2.2. Isolation and differentiation of human monocyte-derived macrophages (HMDM)

Human monocyte-derived macrophages (HMDM) were isolated by standard protocols from buffy coats (35–40 mL) of healthy donors. The study complied with the Declaration of Helsinki and was approved by the Institutional Committee on Human Research at Hospital of Santa Creu i Sant Pau. Cells were applied on 15 mL of Ficoll–Hypaque and centrifuged at 400 × *g* for 40 min at 22 °C, with no brake. Mononuclear cells were obtained from the central white band of the gradient, exhaustively washed in PBS, and resuspended in RPMI medium supplemented with 10% human AB serum, 1% P/S and 1% HEPES. Cells were allowed to differentiate into macrophages by exposure to 10% human AB serum for 7 days, changing the medium every other day. HMDM were arrested, incubated in absence or presence of LPS, washed, and collected for mRNA and protein expression. In some experiments, HMDM were exposed to LPS in absence of presence of native or aggregated LDL.

2.3. Small-interfering RNA (siRNA) gene silencing of LRP1, SREBP-1 and SREBP-2

In brief, HMDM were transfected with siRNA-random (as a control), siRNA-LRP1 (S8278; 50 nM), siRNA-SREBP-1 (S131, 100 nM) and siRNA-SREBP-2 (S28, 100 nM) using siPORT™ Amine in serum-free DMEM (1% glutamine) according to the kit instructions (Silencer™ siRNA Transfection kit; no. 1630). This medium with siRNA was maintained for 48 h. In some experiment, after 48 h, the medium was replaced by a new medium containing LPS and/or native LDL (nLDL) and aggregated LDL (agLDL) (100 μg/mL). After 18 h, cells were exhaustively washed and harvested to test LRP1, SREBP-1 and SREBP-2 mRNA and protein expression. The cells did not take up trypan blue and their morphology was not altered by the procedure.

2.4. Determination of gene expression by real-time PCR

Cell monolayers were washed with cold PBS and total RNA and protein were isolated by using the Tripure™ isolation reagent according to the manufacturer. TaqMan fluorescent real-time PCR primers and probes (6′FAM-MGB) for LRP1 and LDL receptor sequences were designed using a Primer Express software from PE Biosystems and were as follows: LRP1 forward: 5′-gagctgaaccacgcctttg-3′; LRP1 reverse: 5′-ggtagacactgccactccgatac-3′; LRP1 probe: 5′-ttgccatggtgacacag-3′. LDL receptor forward: 5′-tgacaatgtctcaccaagctctg-3′; LDL receptor reverse: 5′-ctcacgctactgggcttcttct-3′: LDL receptor probe: 5′-ctgccagcaacgtcg-3. SREBP-2 mRNA and SREBP-1 mRNA expression were determined using the assays on demand Hs_00190237 and Hs_00231674, respectively. Human 18srRNA (4319413E) was used as endogenous control. TaqMan real-time PCR was performed as previously described [28].

2.5. Western blot analysis

Total protein expression was analyzed by SDS/PAGE as previously described [28]. Blots were incubated with monoclonal antibodies against human LRP1 (β-chain, clone 8B8 RDI 61067, dilution 1:40) or with monoclonal anti-β-actin (clone AC-15) antibodies. The nuclear fraction was obtained following the method described by Dignam et al. [31] with small modifications. Briefly, cells were broken in a Dounce homogenizer (10 strokes) in 10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT and 1% Triton X-100. Nuclei were pelleted by centrifugation for 10 min at 600 × g at 4 °C. The pellets were then resuspended in lysis buffer. Protein expression was analyzed by Western blot analysis and blots were incubated with monoclonal antibodies against human SREBP-1 (clone K-10, dilution 1:100) or SREBP-2 (clone 1D2, dilution 1:5).

2.6. Immunocytochemistry

Human macrophages were seeded onto glass coverslips and then exposed to LPS (100 ng/mL, 18 h). To analyze LRP1, SREBP-1 and SREBP-2 staining, fixed cells were permeated and incubated with primary antibodies against LRP1 (β-chain; clone 8B8 RDI