Atherosclerosis 240 (2015) 424–430

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

FABP4 inhibition suppresses PPARγ activity and VLDL-induced foam cell formation in IL-4-polarized human macrophages

Marcel Boß, Marina Kemmerer, Bernhard Brüne [1], Dmitry Namgaladze [*, 1]

Institute of Biochemistry 1, Faculty of Medicine, Goethe-University Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form
26 February 2015
Accepted 30 March 2015
Available online 3 April 2015

Keywords:
Lipoprotein lipase
Fatty acid binding proteins
PPARγ
Foam cell formation

ABSTRACT

Objective: Macrophages, converted to lipid-loaded foam cells, accumulate in atherosclerotic lesions. Macrophage lipid metabolism is transcriptionally regulated by peroxisome proliferator-activated receptor gamma (PPARγ), and its target gene fatty acid binding protein 4 (FABP4) accelerates the progression of atherosclerosis in mouse models. Since expression of PPARγ and FABP4 is increased upon interleukin-4 (IL-4)-induced macrophage polarization, we aimed to investigate the role of FABP4 in human IL-4-polarized macrophages.

Methods and results: We investigated the impact of FABP4 on PPARγ-dependent gene expression in primary human monocytes differentiated to macrophages in the presence of IL-4. IL-4 increased PPARγ and its target genes lipoprotein lipase (LPL) and FABP4 compared to non-polarized or LPS/interferon γ-stimulated macrophages. LPL expression correlated with increased very low density lipoprotein (VLDL)-induced triglyceride accumulation in IL-4-polarized macrophages, which was sensitive to inhibition of lipolysis or PPARγ antagonism. Inhibition of FABP4 during differentiation using chemical inhibitors BMS309403 and HTS01037 or FABP4 siRNA decreased the expression of FABP4 and LPL, and reduced lipid accumulation in macrophages treated with VLDL. FABP4 or LPL inhibition also reduced the expression of inflammatory mediators chemokine (C-C motif) ligand 2 (CCL2) and IL-1β in response to VLDL in IL-4-polarized macrophages. PPARγ luciferase reporter assays confirmed that FABP4 supports fatty acid-induced PPARγ activation.

Conclusion: Our findings suggest that IL-4 induces a lipid-accumulating macrophage phenotype by activating PPARγ and subsequent LPL expression. Inhibition of FABP4 decreases VLDL-induced foam cell formation, indicating that anti-atherosclerotic effects achieved by FABP4 inhibition in mouse models may be feasible in the human system as well.

1. Introduction

Fatty acid binding proteins (FABPs) constitute a family of intracellular proteins able to bind and transport fatty acids and other lipophilic compounds. Among these, FABP4 (A-FABP, adipocyte-FABP, aP2) and FABP5 (E-FABP, epidermal-FABP, mal1) are expressed both in macrophages and adipocytes, where FABP4 can make up to 5% of total proteins [1]. FABP4 has been reported to support the development of atherosclerosis and insulin resistance in mouse models and its plasma levels are positively correlated with atherosclerotic progression in humans [1–3]. A knockout of FABP4 increased body weight, but also improved insulin sensitivity in mice fed a high fat diet [4]. Inhibition of FABP4 with the inhibitor BMS309403 reduced the negative outcomes of diabetes and atherosclerosis [5]. These data seem to support a deleterious role for FABP4 in atherosclerosis and the metabolic syndrome. However, the mechanisms underlying these effects are not well understood.

FABP4 expression is positively regulated by peroxisome proliferator-activated receptor γ (PPARγ) [6,7]. PPARs are a family of nuclear receptors consisting of PPARα, PPARβ/δ, and PPARγ [8]. These transcription factors regulate metabolic processes such as lipid uptake and oxidation and were also shown to inhibit inflammation [9–11]. PPARγ is the main driver of adipocyte differentiation and is activated by synthetic compounds of the

Abbreviations: CCL, chemokine (C-C motif) ligand; FABP, fatty acid binding protein; LDL, low density lipoprotein; LPL, lipoprotein lipase; M-CSF, macrophage colony-stimulating factor; Mo, monocyte; oxLDL, oxidized LDL; PPAR, peroxisome proliferator-activated receptor; TG, triglyceride; VLDL, very low density lipoprotein.

* Corresponding author.
E-mail address: namgaladze@biochem.uni-frankfurt.de (D. Namgaladze).

[1] These authors share senior authorship.

http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.042
0021-9150/

thiazolidinedione class, such as rosiglitazone [12,13]. Rosiglitazone improves insulin sensitivity, but its use in diabetes treatment may be associated with side effects such as an increased risk of myocardial infarction [14].

Activation of PPARγ and increased expression of its target genes accompanies macrophage polarization after IL-4 stimulation [10,15], which evokes an anti-inflammatory phenotype with increased phagocytosis and immunoregulatory functions [16]. In contrast, stimulation of macrophages with LPS and interferon-γ (IFNγ) elicits a pro-inflammatory phenotype with strong microbicidal and tumoricidal properties and reduces PPARγ expression and activity [17]. In line with increased phagocytosis, the expression of scavenger receptor CD36 is induced in IL-4-polarized macrophages supporting foam cell formation in cells exposed to saturated fatty acids or modified lipoprotein particles [18,19]. PPARγ also regulates the expression of lipoprotein lipase (LPL) [20] that supports the uptake of fatty acids from very low density lipoproteins (VLDL) into macrophages by lipolysis of VLDL particles and therefore provokes triglyceride (TG)-rich foam cell formation [21]. In addition, LPL fosters the uptake of lipoprotein particles by facilitating their cell surface binding [22]. If the expression of PPARγ during monocyte differentiation is inhibited by 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), macrophages show decreased foam cell formation [23]. This is consistent with a role of PPARγ in promoting the expression of genes handling macrophage lipid uptake.

The interaction of FABP4 and PPARγ has been addressed in several publications. Some studies suggested that FABP4 transports ligands to PPARγ and therefore enhances PPARγ activity [24,25], whereas others report the opposite and imply a ligand sequestering function of FABP4 [26]. Recently, FABP4 was found to bind PPARγ and to induce its ubiquitination and subsequent degradation [27]. In this study we show that IL-4-induced FABP4 expression augments PPARγ activation and therefore amplifies the expression of LPL in IL-4-polarized macrophages, which causes increased TG accumulation in macrophages.

2. Methods

2.1. Isolation of monocytes from human blood

Human monocytes were isolated from buffy coats of anonymous donors (DRK-Blutspendedienst Baden-Württemberg-Hessen, Frankfurt am Main, Germany) using Ficoll density centrifugation followed by magnetic separation of CD14-positive cells with magnetic beads (Miltenyi-Biotec). Cells were seeded in macrophage-SFM complete medium (Life Technologies) supplemented with 100 U/ml penicillin (PAA), 100 μg/ml streptomycin (PAA) and differentiated to macrophages with 50 ng/ml macrophage colony-stimulating factor (M-CSF; Immunotools). Cells were either additionally polarized with 100 U/ml interferon γ and 100 ng/ml LPS or 20 ng/ml IL-4 (Immunotools). After 3 d of differentiation and polarization macrophages were used for the experiments. This investigation conforms to the ethical principles outlined in the Declaration of Helsinki and was approved by the university ethics committee (Ethik-Kommission des Fachbereichs Medizin der Goethe-Universität Frankfurt am Main). The ethics committee waived the need for consent when using commercially available blood of anonymous donors.

2.2. siRNA-mediated FABP4 knockdown

FABP4 knockdown by siRNA was done using electroporation with the Amaxa Human Monocyte Nucleofector Kit (Lonza) in a Nucleofector device according to manufacturer's instructions. 3×10^6 CD14-positive cells were electroporated with 100 nM (i. e. 10 pmol/sample) siRNA for FABP4 (siGENOME Human FABP4 SMARTpool, Dharmacon) or control siRNA. The cells were cultured as described above after electroporation.

2.3. RNA isolation and analysis

Total RNA was isolated using PeqGold RNAPure (Peqlab) according to manufacturer's instructions and was transcribed to cDNA using Maxima First Strand cDNA synthesis kit (Thermo Scientific), following manufacturer's instructions. Quantitative PCR was performed with iQ SYBR green Supermix (Bio-Rad) using a CFX96 (Bio-Rad) system. Primer sequences can be obtained upon request. Expression was normalized to β2 microglobulin (βMG) or TATA box-binding protein (TBP) RNA.

2.4. VLDL and LDL isolation

VLDL and LDL isolation was performed as described elsewhere [28]. Briefly, human VLDL and LDL of healthy donors (DRK-Blutspendedienst Baden-Württemberg-Hessen, Frankfurt am Main, Germany) was isolated from the plasma fraction by sequential ultracentrifugation and maintained in the presence of 0.2 mM EDTA to prevent oxidation. Oxidized LDL (oxLDL) was prepared by incubating LDL with 5 μM $CuSO_4$ at room temperature for 24 h followed by dialysis against PBS with 100 μM EDTA [29].

2.5. Triglyceride determination

Before triglyceride content determinations, macrophages were treated with 20 μg/ml VLDL for 24 h. Cells were subsequently lysed in PBS supplemented with 1% Triton X-100. After sonication and centrifugation (10 min, 10,000 g, 4 °C) the triglyceride content of the lysate was measured with the triglyceride kit (Roche) according to manufacturer's instructions. Triglyceride content was normalized to protein concentration in the lysates.

2.6. DiI-oxLDL uptake

To label oxLDL with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), oxLDL was incubated with 10 μM DiI at 37 °C overnight. The next day, precipitated DiI was removed by filtration through a 0.22 μm filter. Macrophages were stimulated with 20 μg/ml DiI-oxLDL for 6 h. The uptake was stopped by adding ice-cold PBS. Cells were scraped and PE561 fluorescence was analyzed by flow cytometry. Median fluorescence of at least 10,000 events was recorded.

2.7. Cloning of a FABP4 overexpressing plasmid

Cloning of the human FABP4 overexpression plasmid was performed using SBI System Biosciences, Clone-it Enzyme free Lentivectors (pCDH-EF1-MCS-T2A), according to the manufacturer's protocol. Briefly, isolated total DNA of human primary macrophages served as a template in PCR using High Fidelity DNA Polymerase (Roche). The following primers were used: FABP4-fwd: 5′-ATGTGTGATGCTTTTGTAGGTACCT-3′; FABP4-rev: 5′-TGCTCTCTCA-TAAACTCTCGTGG-3′. PCR products were cleaned by QIAquick PCR Purification Kit (Qiagen) and annealed by heating the mix at 95 °C and slowly cooling down. Following transformation resulting clones were verified by sequencing.

2.8. PPARγ reporter assay

HEK293T cells were cultured in DMEM High Glucose medium