Atherosclerosis 217 (2011) 106–112

Contents lists available at ScienceDirect

Atherosclerosis

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

Scavenger receptor SR-BI in macrophage lipid metabolism

Ailing Ji [b,e,f], Jason M. Meyer [b,c,e], Lei Cai [b,e,f], Akinwunmi Akinmusire [f], Maria C. de Beer [b,d,e,f], Nancy R. Webb [b,e,f], Deneys R. van der Westhuyzen [a,b,c,e,f,*]

[a] Department of Veterans Affairs Medical Center, Lexington, KY, United States
[b] Department of Internal Medicine, University of Kentucky, Lexington, KY 40536, United States
[c] Department of Molecular and Cellular Biochemistry University of Kentucky, Lexington, KY 40536, United States
[d] Department of Physiology, University of Kentucky, Lexington, KY 40536, United States
[e] Cardiovascular Research Center, University of Kentucky, Lexington, KY 40536, United States
[f] Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY 40536, United States

ARTICLE INFO

Article history:
Received 6 August 2010
Received in revised form 7 March 2011
Accepted 11 March 2011
Available online 9 April 2011

Keywords:
SR-BI
ABCA1
ABCG1
Cholesterol efflux
Macrophage
HDL

ABSTRACT

Objective: To investigate the mechanisms by which macrophage scavenger receptor BI (SR-BI) regulates macrophage cholesterol homeostasis and protects against atherosclerosis.
Methods and results: The expression and function of SR-BI was investigated in cultured mouse bone marrow-derived macrophages (BMM). SR-BI, the other scavenger receptors SRA and CD36 and the ATP-binding cassette transporters ABCA1 and ABCG1 were each distinctly regulated during BMM differentiation. SR-BI levels increased transiently to significant levels during culture. SR-BI expression in BMM was reversibly down-regulated by lipid loading with modified LDL; SR-BI was shown to be present both on the cell surface as well as intracellularly. BMM exhibited selective HDL CE uptake, however, this was not dependent on SR-BI or another potential candidate glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1). SR-BI played a significant role in facilitating bidirectional cholesterol flux in non lipid-loaded cells. SR-BI expression enhanced both cell cholesterol efflux and cholesterol influx from HDL, but did not lead to altered cellular cholesterol mass. SR-BI-dependent efflux occurred to larger HDL particles but not to smaller HDL_3. Following cholesterol loading, ABCA1 and ABCG1 were up-regulated and served as the major contributors to cholesterol efflux, while SR-BI expression was down-regulated.
Conclusion: Our results suggest that SR-BI plays a significant role in macrophage cholesterol flux that may partly account for its effects on atherogenesis.

Published by Elsevier Ireland Ltd.

1. Introduction

Class B Scavenger Receptor Type 1 (SR-BI) is a CD36-related cell surface glycoprotein involved in lipid metabolism [1,2]. SR-BI is recognized mainly as an HDL receptor, which mediates selective uptake of HDL cholesteryl esters (CE) into cells. SR-BI is most abundantly expressed in liver and steroidogenic tissues, where it functions to deliver HDL CE for cholesterol excretion and for steroid hormone synthesis, respectively [3,4]. SR-BI can also facilitate cholesterol efflux from cells including macrophages [5]. In addition to its roles in cholesterol metabolism, other important functions for SR-BI have been proposed, such as platelet aggregation [6], oxidative stress [7], endothelial nitric oxide synthase activation [8] and apoptosis [9]. Hepatic SR-BI plays a pivotal role in HDL cholesterol clearance from plasma and consequently plasma HDL cholesterol levels [10]. Hepatic SR-BI expression is also an important positive regulator of the rate of macrophage-to-feces reverse cholesterol transport (RCT) [11]. In line with this concept, SR-BI has been shown to play an anti-atherogenic role in mice [12–14].

Studies have shown that liver specific SR-BI null mice develop less atherosclerosis than that from whole body SR-BI null mice, suggesting an atheroprotective function of SR-BI in peripheral tis-

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; acLDL, acetylated low density lipoprotein; apoA-I, apolipoprotein A-I; BMM, bone marrow-derived macrophage; CE, cholesteryl ester; GPIHBP1, glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1; HDL, high density lipoprotein; LCM, L-cell conditioned medium; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum; MPM, mouse peritoneal macrophage; SRA, class A scavenger receptor; SR-BI, class B scavenger receptor type 1.

* Corresponding author at: University of Kentucky, CTW Health Sciences Building, Room 541, 900 S. Limestone Street, Lexington, KY 40536, United States.
Tel.: +1 859 323 4933x81397; fax: +1 859 257 3646.
E-mail addresses: ailing.ji@uky.edu (A. Ji), jmmeye3@uky.edu (J.M. Meyer), lcai8@uky.edu (L. Cai), asakin2@uky.edu (A. Akinmusire), mdebeer@uky.edu (M.C. de Beer), nrwebb1@uky.edu (N.R. Webb), dvwest1@uky.edu (D.R. van der Westhuyzen).

0021-9150/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2011.03.017

sues [15]. SR-BI is detected in cultured human monocyte-derived macrophages and atherosclerotic lesions [16]. An athero-protective role of macrophage SR-BI has been suggested from studies performed using bone marrow transplantation. Inactivation of macrophage SR-BI promotes advanced atherosclerotic lesion development in apolipoprotein E-deficient mice [17], as well as in LDL receptor-deficient mice [18,19]. However, macrophage SR-BI may have dual effects on atherogenesis in that it is reported to promote early lesion development while inhibiting more advanced lesions [19]. The mechanisms by which macrophage SR-BI modulate atherosclerosis are unclear.

Macrophage foam cell formation with CE accumulation plays a key role in the formation of atherosclerotic lesions. Cholesterol efflux from macrophages, mediated by the ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1) plays a key role in preventing foam cell formation. On the other hand, the role of SR-BI in macrophage cholesterol efflux is uncertain. In bone marrow transplantation studies, one study reported that SR-BI plays a significant role in efflux from peritoneal macrophages (MPM) [19], while a second study failed to show an SR-BI effect on efflux [17]. Another study in which cholesterol efflux to human serum was measured, indicated a minimal role for macrophage SR-BI in efflux [20]. Previous studies have demonstrated that SR-BI stimulates the bidirectional flux of free cholesterol between HDL and SR-BI-expressing cells rather than efflux alone [21]. To investigate potential functions of SR-BI in macrophage lipid metabolism, the regulation of SR-BI expression and other cholesterol transporters as well as their roles in cholesterol transport were studied during the differentiation of bone marrow-derived macrophages (BMM). Our results suggest that SR-BI plays a significant role in macrophage cholesterol flux that may contribute to the protective effects in atherogenesis.

2. Materials and methods

2.1. Animals

SR-BI-deficient mice (SR-BI-null) were obtained from M. Krieger [4]. SR-BI homozygous (*SR-BI−/−*) and wild type (WT) mice (both 1:1 mixed C57BL/6× 129 backgrounds) were bred from a common mating pair of SR-BI heterozygous (*SR-BI+/−*) mice. ABCG1-deficient mice (ABCG1-null) from Deltagen were backcrossed to a C57BL/6 background (>99.9% C57BL/6 background). For all animal experiments, 8- to 12-week-old male mice, weighing 20–25 g, were used. All animal experiments were approved by the Veterans Affairs Medical Center, Institutional Animal Care and Use Committee.

2.2. Bone marrow macrophage (BMM) preparation

Murine BMM were obtained and cultured by standard procedures [22]. Isolated cells were suspended and cultured in RPMI 1640 containing 50 IU of penicillin G per ml, 50 μg of streptomycin per ml, 2 mM glutamine, 10% fetal bovine serum, and 15% (vol/vol) L-cell conditioned medium (LCM).

2.3. Flow cytometry

Flow cytometric analysis of BMM cell-surface marker expression was carried out as described by Yona et al. [22]. Attached cells were dissociated by incubation at room temperature with an enzyme-free cell dissociation buffer (Gibco) for 25 min. Trypan blue positivity was less than 5% for all samples. Cells were stained with rat anti-mouse CD11b (Mac1, Invitrogen), or F4/80 (AbD Serotec) or control rat IgG, followed by FITC-conjugated chicken anti-rat IgG antibody (Molecular Probes). Flow cytometry was performed using a FACScalibur cytometer (Becton Dickinson, Cowley, U.K.).

2.4. Western blotting

Total cell proteins (10 μg) were separated on a 4–20% polyacrylamide gradient gel, transferred to PVDF membranes and immunoblotted with rabbit anti-mouse SR-BI (Novus, NB400-104), rat anti-mouse CD36 [23], goat anti-mouse SRA (R&D Systems, AF1797), mouse anti-human ABCA1 (gift from M. Hayden), rabbit anti-mouse ABCG1 (Novus, NB400-132), or mouse anti-β-actin (Sigma, A5441). Immunoblots were visualized by the Amersham™ ECL™ Western Blotting Detection Reagents (GE Healthcare).

2.5. Cellular lipid and protein determinations

Lipids were extracted as described previously [24] and aliquots were assayed for total and free cholesterol content using a colorimetric kit (Wako). Cell protein was determined using BCA™ Protein Assay Kit (Thermo Scientific).

2.6. Biotinylation of cell surface SR-BI

Biotinylation of cell surface proteins was carried out using EZ-Link Sulfo-NHS-LC-Biotin (Thermo Scientific) as described previously [25] followed by isolation using Streptavidin Iron Oxide Particles (Sigma). The supernatant (intracellular proteins) and the pellet (surface proteins) were analyzed by SDS-PAGE and immunoblotting using the rabbit anti-mouse SR-BI (Novus, NB400-104) antibody and quantified by densitometry.

2.7. Isolation and labeling of lipoproteins

HDL (d = 1.063–1.21 g/mL) was isolated from C57BL/6 mouse plasma or human plasma by density gradient ultracentrifugation [26]. Human HDL was subfractionated to obtain HDL_{2b} (d = 1.09–1.11 g/mL) and HDL_3 (d = 1.13–1.18 g/mL). Protein concentrations were determined by the method of Lowry et al. [27]. HDL_{2b} was radiolabeled by the iodine monochloride method and HDL-associated CE was traced with non-hydrolyzable [1,2(n)-^{3}H]cholesteryl oleoyl ether (Amersham Biosciences) [25]. Lipoproteins were analyzed by SDS-PAGE and non-denaturing gradient gel electrophoresis. LDL (d = 1.019–1.063 g/mL) was isolated from human plasma [26]. Acetylated LDL (acLDL) was prepared as described previously [28].

2.8. HDL cell binding, association and selective uptake

HDL cell association, selective CE uptake and 4 °C binding assays were performed as described previously [25]. BMM were incubated with ^{125}I/[^{3}H]CEt labeled HDL_{2b} (10 μg/mL) in serum-free medium containing 0.5% fatty acid-free BSA for 4 h at 37 °C. Cell association of ^{125}I-HDL protein uptake was determined. The non-iodide, trichloroacetic acid-soluble ^{125}I in cell medium corresponding to degraded ^{125}I-apolipoprotein was assayed. Selective lipid uptake is defined as ^{3}H – (^{125}I cell-associated + ^{125}I degraded) and represents the uptake of cholesteryl ester that cannot be accounted for by the internalization of intact particles. SR-BI-specific values were calculated as the difference between the values for WT and SR-BI-null cells.

2.9. RT-PCR

PCR amplification of mouse glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) was carried out using the following gene specific primers: forward

Download English Version:

https://daneshyari.com/en/article/5949559

Download Persian Version:

https://daneshyari.com/article/5949559

Daneshyari.com