Biochimica et Biophysica Acta 1813 (2011) 1917–1924

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

PLTP regulates STAT3 and NFκB in differentiated THP1 cells and human monocyte-derived macrophages

S. Vuletic [a], W. Dong [a,1], G. Wolfbauer [a], C. Tang [b], J.J. Albers [a,*]

[a] Northwest Lipid Metabolism and Diabetes Research Laboratories, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA
[b] Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA

ARTICLE INFO

Article history:
Received 20 June 2011
Accepted 27 June 2011
Available online 3 July 2011

Keywords:
STAT3 activation
NFκB reduction
Inflammation
Human monocyte-derived macrophage
Differentiated THP1 cell

ABSTRACT

Phospholipid transfer protein (PLTP) plays an important role in regulation of inflammation. Previously published studies have shown that PLTP binds, transfers and neutralizes bacterial lipopolysaccharides. In the current study we tested the hypothesis that PLTP can also regulate anti-inflammatory pathways in macrophages. Incubation of macrophage-like differentiated THP1 cells and human monocyte-derived macrophages with wild-type PLTP in the presence or absence of tumor necrosis factor alpha (TNFα) or interferon gamma (IFNγ) significantly increased nuclear levels of active signal transducer and activator of transcription 3, $pSTAT3_{Tyr705}$ ($p<0.01$). Similar results were obtained in the presence of a PLTP mutant without lipid transfer activity ($PLTP_{M159E}$), suggesting that PLTP-mediated lipid transfer is not required for activation of the STAT3 pathway. Inhibition of ABCA1 by chemical inhibitor, glyburide, as well as ABCA1 RNA inhibition, reversed the observed PLTP-mediated activation of STAT3. In addition, PLTP reduced nuclear levels of active nuclear factor kappa-B (NFκB) p65 and secretion of pro-inflammatory cytokines in conditioned media of differentiated THP1 cells and human monocyte-derived macrophages. Our data suggest that PLTP has anti-inflammatory capabilities in macrophages.

1. Introduction

Macrophages are the key immune cells in organs and tissues, are involved in the innate immune response, and play a critical role in both initiation and resolution of inflammation. Activation of the macrophage-dependent immune response is of decisive importance in acute infections. However, prolonged activation of macrophages associated with chronic disease is often detrimental, and contributes to the pathophysiological processes of numerous chronic diseases, including atherosclerosis, chronic kidney disease and diabetes. Therefore, understanding the physiological and pathophysiological processes that regulate the macrophage-dependent immune response is a significant element in the formulation of novel prophylactic and therapeutic approaches for many chronic diseases.

Phospholipid transfer protein (PLTP) is a versatile, widely expressed protein involved in transport of lipids and vitamin E among lipoproteins, and between lipoproteins and cells. Reported functional roles of PLTP include participation in reverse cholesterol transfer, lipoprotein metabolism, inflammatory processes, signal transduction, cell differentiation and apoptosis [1–8]. PLTP levels and activity are significantly altered in numerous human diseases and conditions with a strong inflammatory component, such as atherosclerosis, diabetes, and neuroinflammatory diseases [9–16]. These reports suggest that PLTP may be playing an important role in the regulation of inflammatory processes, although the mechanisms of PLTP actions are currently poorly understood.

PLTP is expressed in macrophages in both normal and atherogenic blood vessels [9,17,18]. Recent reports suggest that macrophage-derived PLTP plays an important role in atherosclerosis [19,20]. However, the interplay between macrophages and PLTP is inadequately understood. The macrophage-dependent immune response is regulated by signal transduction pathways dependent on extracellular ligands and other pathway components that activate or inactivate signal transduction processes. We previously reported that PLTP interacts with the ATP-binding cassette A1 (ABCA1) to enhance cholesterol efflux from cells, and that PLTP's interaction with ABCA1 activates Janus kinase-2, JAK2 [21,22]. In this study we provide evidence that PLTP activates STAT3, and inhibits NFκB in macrophage-like THP1 cells and in human monocyte-derived macrophages.

Abbreviations: ABCA1, ATP-binding cassette transporter A1; IFNγ, interferon gamma; IL1α, interleukin 1 alpha; JAK2, Janus kinase-2; LPS, lipopolysaccharides; MIP1α, macrophage inflammatory protein 1 alpha; NFκB, nuclear factor kappa-B; PLTP, phospholipid transfer protein; PMA, phorbol 12-myristate 13-acetate; STAT3, signal transducer and activator of transcription 3; TBP, TATA binding protein; TGFβ, tumor growth factor beta; TLR, Toll-like receptor; TNFα, tumor necrosis factor alpha

* Corresponding author at: University of Washington, NWRL, 401 Queen Anne Ave. N, Seattle, WA 98109, USA. Tel.: +1 206 685 3330; fax: +1 206 685 3279.
E-mail addresses: simona@uw.edu (S. Vuletic), dongwj@mail.xjtu.edu.cn (W. Dong), gwolfb@uw.edu (G. Wolfbauer), crtang@uw.edu (C. Tang), jja@uw.edu (J.J. Albers).
[1] Present address: Xi'an Jiaotong University School of Medicine, Department of Human Anatomy and Histology and Embryology, Xi'an 710061, People's Republic of China.

0167-4889/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2011.06.013

2. Materials and methods

2.1. Antibodies, cells, and materials

Antibodies against phosphorylated JAK2 (Cat. No. 3771), STAT3 (Cat. No. 4904), STAT3 phosphorylated at tyrosine 705 (Cat. No. 9131), NFκB p65 total and phosphorylated at serine 536 (Cat. Nos. 3034 and 3033, respectively), IL1β (Cat. No. 2022), TGFβ (Cat. No. 3711), and human TNFα (Cat. No. 2169) were obtained from Cell Signaling Technology (Danvers, MA). Antibody against JAK2 was obtained from Santa Cruz Biotechnologies (Cat. No. SC-34479-R) TATA-binding protein (TBP) and MIP1α antibodies (Cat. Nos. 62125; and ab0381, respectively) were from Abcam (Cambridge, MA). Anti-IL6 (Cat. No. AF-206-NA) and anti-IL10 antibodies (Cat. No. AF-217-NA), as well as IL1β, MIP1α and TGFβ ELISA plates (Cat. Nos. HSLB00C, DMA00 and DB100B, respectively) were purchased from R&D Systems (Minneapolis, MN). Antibody against β-actin (Cat. No. A2066), interferon gamma (IFNγ; Cat. No. I32265), PMA (Cat. No. P8139) and glyburide (Cat. No. G2569) were from Sigma-Aldrich (St. Louis, MO). Secondary antibodies, anti-rabbit, anti-mouse and anti-goat TrueBlot (Cat. Nos. 18-8816; 18-8817; and 18-8814, respectively), and recombinant human M-CSF and GM-CSF (Cat. Nos. 14-8789 and 14-8339, respectively) were from eBioscience Inc. (San Diego, CA). Human ABCA1 shRNA Mission lentiviral transduction particles and control Mission pLKO.1-puro transduction particles were from Sigma-Aldrich St. Louis, MO (Cat. Nos. SHCLNV-NM_005502 and SHC001V, respectively), as well as puromycin (Cat. No. P9620). Protein isolation kit, NE-PER, HALT protease inhibitor cocktail, Detoxi-Gel endotoxin removing column (Cat. No. 20344) and SuperSignal West Femto maximum sensitivity substrate were obtained from Thermo Scientific/Pierce Biotechnology (Rockford, IL). Phosphatase inhibitor cocktail (Cat. No. 524627) and PhosphoSafe protein isolation solution were purchased from Calbiochem-EMD BioSci (La Jolla, CA). Criterion XT gels, MOPS buffer and XT sample buffer were obtained from Bio-Rad (Hercules, CA). PhosphoBlocker blocking reagent (Cat. No. AKR-104) was from Cell Biolabs (San Diego, CA). Human mononuclear cells PBMC (Cat. No. 1001) were procured from Astarte Biologics (Redmond, WA). Specialized macrophage medium, macrophage-SFM, was purchased from Invitrogen (Carlsbad, CA). RPMI-1640 media, L-glutamine, non-essential amino-acids, and trypsin–versene were obtained from Lonza Biosciences (Walkersville, MD). Nutridoma-SP (Cat. No. 11011375001) was from Roche Applied Science (Indianapolis, IN). Characterized fetal bovine serum with endotoxin levels below biologically significant concentration was acquired from Thermo Fisher Scientific/Hyclone (Logan, UT). [C^{14}]-labeled phosphatidylcholine was purchased from Perkin Elmer Life Sci. Pyrochrome *Limulus polyphemus* Amoebocyte Lysate (LAL) kit (Cat. No. C1500), endotoxin standards (*E. coli* O113-H10; Cat. No. EC010), LAL reagent water, Pyroplate microplates, endotoxin-free pipette tips and borosilicate glass tubes were from the Associates of Cape Cod Inc. (East Falmouth, MA). Multiplex kits for human cytokines and chemokines were purchased from Linco Research (St. Charles, MO) and Bio-Rad (Hercules, CA). Recombinant wild-type and mutant PLTP were expressed and isolated as previously reported [1,23].

2.2. Cell culture

The human monocytic leukemia cell line THP1 (ATCC Cat. No. TIB-202) was grown in RPMI media supplemented with 10% fetal bovine serum or Nutridoma, at 37 °C in 5% CO_2. No differences were observed in cells grown with Nutridoma compared to serum supplementation (not shown). THP1 cells in suspension were seeded at equal density into multiwell plates, differentiated into macrophage-like cells by addition of PMA (1.6×10^{-7} M) for 72 h, and then used in experiments. These THP1 cells were pre-incubated with PLTP (5 μg/ml) for 4 h, extensively washed with PBS, and then exposed to either 100 ng/ml LPS, 10 ng/ml IFNγ, or 100 ng/ml TNFα for up to 24 h. Similar studies were performed using co-incubation protocols. In time-dependent studies for both modalities (pre- and co-incubation) the optimal time for our experiments was evaluated. Chosen protocols were confirmed by experiments under identical conditions using fully developed human monocyte-derived macrophages 8 days after attachment, which had been grown in Macrophage-SFM medium, supplemented with GM-CSF. The cells were pre-incubated with PLTP in SFM medium for 4 h, extensively washed and then incubated for up to 24 h with TNFα. The resulting conditioned media had reduced IL-1β levels, indicating that pre-incubation of human monocyte-derived macrophages with PLTP reduces secretion of pro-inflammatory cytokines similar to THP1 cells (see Results), thus validating our cell culture data obtained in the THP1 cell line. All cell culture experiments were performed in triplicate for each condition, and repeated at least three times to confirm our results.

2.3. ABCA1 RNA inhibition

Inhibition of ABCA1 expression in THP1 cells was performed using human ABCA1 shRNA Mission lentiviral transduction particles (five constructs: TRCN0000029089, TRCN0000029090, TRCN0000029091, TRCN0000029092 and TRCN0000029093) and control Mission pLKO.1-puro transduction particles (Sigma-Aldrich, St. Louis, MO). We initially established the concentration of the selection agent (puromycin) required to kill 100% of THP1 cells. Cells were incubated for 48 h in growth medium without (control) or with puromycin (0.1–1 μg/ml). Cell survival was assessed by Trypan blue. All cells were dead in the presence of 0.6 μg/ml of puromycin, and this concentration of puromycin was used in the selection protocol.

Transduction of THP1 cells was performed by spinoculation (transduction by centrifugation; modified from [24]). THP1 cells (3×10^5 cells/ml) grown in suspension in RPMI medium supplemented with 10% FBS were placed in sterile conical 15 ml tubes (6 tubes; 2 ml of cells in each tube). Lentiviral particles (11 μl/ml of cells) were added to the cells and centrifuged at 1000 ×*g*, 28 °C for 1 h. Medium containing lentiviral particles was aspirated, cells mixed with fresh growth medium (RPMI, 10% FBS) and placed in a 6-well plate. Cells were incubated at 37 °C, 5% CO_2, 95% humidity for 48 h. Following incubation, the spinoculation was repeated and cells placed in puromycin-containing medium (0.6 μg/ml) for selection of clones containing puromycin-resistant constructs. Control THP1 cells were subjected to the same procedure, but without exposure to the virus or puromycin. Cell viability was tested after 48 h, and all samples contained viable cells. Clones were expanded in the presence of puromycin, and tested for ABCA1 by qRT-PCR and Western blotting. Based on these studies, THP1 cells in which ABCA1 was inhibited with construct TRCN0000029089 were selected for analysis.

Following successful transduction, spinoculation control THP1 cells, THP1 cells containing control (mock) or ABCA1 shRNA construct were incubated with PMA for 72 h in the presence of puromycin. Adherent THP1 macrophages were used for evaluation of the PLTP effect on activation of STAT3.

2.4. Protein isolation

Cytoplasmic and nuclear proteins were isolated using the NE-PER kit according to the manufacturer's instructions, with addition of phosphatase inhibitor cocktail to preserve phosphorylated proteins. Membrane proteins were isolated using MEM-PER kit according to the manufacturer's instructions. Whole cell lysates were prepared using PhosphoSafe protein isolation solution, according to the manufacturer's instructions. Samples were evaluated for total protein concentration, and aliquots stored at −70 °C until analysis.