Atherosclerosis 216 (2011) 292–298

Contents lists available at ScienceDirect

Atherosclerosis

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

Oncostatin M is expressed in atherosclerotic lesions: A role for Oncostatin M in the pathogenesis of atherosclerosis

Adaia Albasanz-Puig[a,1], Jacqueline Murray[a], Michael Preusch[b,2], Daniel Coan[a], Mayumi Namekata[a], Yatin Patel[c], Zhao Ming Dong[a,b], Michael E. Rosenfeld[b], Errol S. Wijelath[a,*]

[a] Department of Surgery, Division of Vascular Surgery, VA Puget Sound Health Care System and the University of Washington School of Medicine, Seattle, WA, USA
[b] Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA
[c] Department of Cardiology, Cardiovascular Division, Kings College London School of Medicine London, UK

ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 19 January 2011
Accepted 1 February 2011
Available online 3 March 2011

Keywords:
Oncostatin M
Smooth muscle cell
Atherosclerosis
Carotid plaques
ApoE$^{-/-}$
STAT-1
STAT-3

ABSTRACT

Objective: Chronic inflammation plays a pivotal role in the development and progression of atherosclerosis. The inflammatory response is mediated by cytokines. The aim of this study was to determine if Oncostatin M (OSM), a monocyte and T-lymphocyte specific cytokine is present in atherosclerotic lesions. We also investigated the roles of signal transducer and activator of transcription (STAT)-1 and STAT-3 in regulating OSM-induced smooth muscle cell (SMC) proliferation, migration and cellular fibronectin (cFN) synthesis.

Methods and results: Immunostaining of atherosclerotic lesions from human carotid plaques demonstrated the expression of OSM antigen in both macrophages and SMCs. Explanted SMCs from human carotid plaques expressed OSM mRNA and protein as determined by RT-PCR and Western blotting. Using the chow-fed ApoE$^{-/-}$ mouse model of atherosclerosis, we observed that OSM was initially expressed in the intima at 20 weeks of age. By 30 weeks, OSM was expressed in both the intima and media. In vitro studies show that OSM promotes SMC proliferation, migration and cFN synthesis. Lentivirus mediated-inhibition of STAT-1 and STAT-3 prevented OSM-induced SMC proliferation, migration and cellular fibronectin synthesis.

Conclusions: These findings demonstrate that OSM is expressed in atherosclerotic lesions and may contribute to the progression of atherosclerosis by promoting SMC proliferation, migration and extracellular matrix protein synthesis through the STAT pathway.

Published by Elsevier Ireland Ltd.

1. Introduction

It is now recognized that chronic inflammation plays a key role in the initiation and progression of atherosclerotic lesions [1]. The inflammatory response is mediated by cytokines released by infiltrating macrophages and T-lymphocytes. In addition, cells within the lesion such as smooth muscle cells (SMCs) and endothelial cells can also synthesize and secrete cytokines [2]. If unchecked, the inflammatory response will promote cell proliferation, migration and matrix synthesis which are the major factors that contribute to narrowing of the vessel lumen [3]. Many inflammatory cytokines upon binding to their receptors exert their effects through activation of the Janus kinase and signal transducer and activator of transcription signaling pathway (JAK/STAT). Published data suggests that the JAK/STAT plays an important role in the development of cardiovascular diseases [4–6]. In particular, activation of the JAK/STAT pathway by cytokines signaling through the glycoprotein 130 (gp130) receptor subunit has been linked to the inflammatory response associated with vascular diseases [7]. The gp130 cytokine interleukin-6 (IL-6), is not only a biological marker for vascular inflammation, but also participates in the inflammatory process. Other members of the gp130 cytokine family include Oncostatin M (OSM), leukemia inhibitory factor, IL-11, IL-27, cardiotrophin 1 and ciliary neurotrophic factor [8].

We have previously demonstrated that OSM, a macrophage and T-lymphocyte specific cytokine, was able to induce prolonged tissue factor expression in SMCs [9]. More recently, Kastl et al. demonstrated that thrombin was able to stimulate synthesis of OSM from human carotid plaque macrophages [10]. These observations suggest a potential role for OSM in promoting a thrombogenic state in atherosclerotic lesions. OSM has also been

* Corresponding author at: Department of Veterans Affairs Medical Center, 1660, S Columbian Way, Seattle, WA 98108, USA.
Tel.: +1 206 277 1188; fax: +1 206 277 4483.
E-mail address: errolw@u.washington.edu (E.S. Wijelath).
[1] Present address: Vall d'Hebrón Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain.
[2] Present address: Department of Cardiology, University of Heidelberg, Heidelberg, Germany.

0021-9150/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2011.02.003

shown to stimulate the synthesis of matrix metalloproteinase, monocyte chemoattractant protein-1, alkaline phosphatase and urokinase plasminogen activator [11–14]. Collectively, these observations suggest a potential role for OSM in modulating the pathophysiologic processes underlying cardiovascular diseases.

The purpose of our study was to determine whether OSM was expressed in atherosclerotic lesions and to investigate the role of OSM in promoting SMC proliferation, migration and cellular fibronectin (cFN) synthesis. We also investigated the transduction signals that control OSM biological effects.

2. Methods

2.1. Human subjects

Whole excised intact carotid plaques were obtained from 16 patients undergoing carotid endarterectomy according to protocols approved by the Veterans Administration Institutional Review Board. Informed consent was obtained from all patients. Specimens from each patient were divided into two equal sections. One section was fixed in 10% buffered formalin, dehydrated and embedded in paraffin for immunohistochemical analysis. The other section was used for isolation of SMCs.

2.2. $ApoE^{-/-}$ mice

All animal studies were approved by the University of Washington Institutional Animal Care and Use Committee in accordance with the guidelines set forth by the NIH Guide for the Care and Use of Laboratory Animals. Chow-fed mice were euthanized at 20, 30 and 54 weeks of age, and perfusion fixed with formalin at physiological pressure via the left ventricle. The innominate artery (also called the brachiocephalic trunk, a small segment at the base of the right carotid artery) was removed and embedded in paraffin.

2.3. Immunohistochemical analysis

Serial 5 μm thick paraffin sections from human carotid plaques and $ApoE^{-/-}$ mice innominate arteries were stained with antibodies to OSM (clone N-1 for human OSM from Santa Cruz, and mouse OSM from R&D Systems), SMC-α actin, vWF and CD68 (DAKO). To quantify vWF stained microvessels in atherosclerotic lesions, three high-powered fields (HPF) ×400 were chosen and the average number of microvessels was obtained. Sections were developed using the Immpress reagent and Impact DAB (Vector labs) kits and counterstained with hematoxylin. Controls consisted of normal IgG or isotype matched monoclonal antibodies.

2.4. Culture of SMCs from carotid plaques

Carotid plaques were minced into approximately 2 mm fragments, placed in 35 mm gelatin-coated plates and maintained in SMC growth medium (Cell Applications). Outgrowth of plaque and medial SMCs was observed after 1 week. RNA and protein was extracted from SMCs after 3 passages for analysis of OSM mRNA and protein. Normal human aortic and carotid SMCs (Cell Applications) were maintained in SMC growth medium. Cells were used between passages 3 and 6.

2.5. Quantitative real-time PCR

Total RNA was extracted using RNeasy kit (Qiagen). RNA (0.25 μg) was treated with DNase-1 and cDNA synthesized using the RT^2 EZ first strand kit (SABiosciences). We used quantitative real-time PCR (qPCR) to analyze the expression levels of STAT-1, STAT-3 and the splice variant forms of EDA and EDB cFN. qPCR (10 ng cDNA) was performed using the SYBR green RT^2 qPCR master mix (SABiosciences) according to the manufacturers' instructions. Each sample was analyzed in duplicate. The house-keeping gene RPL13A was used for normalization. RPL13A gene was selected after validation for expression stability against a panel of house-keeping genes. Relative gene expression was calculated by using the $\Delta\Delta C_T$ method [15].

For amplification of OSM and RPL13A, cDNA (50 ng) was amplified using the following parameters: denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 15 s, then 60 °C for 30 s. PCR products were run on 2% TAE agarose gels followed by staining with ethidium bromide. The primer sequences used for PCR were as follows: *OSM forward:* 5′-CATCGAGGACTTGGAGAAGC-3′, *OSM reverse:* 5-TCAGCCGTGTCTGAGTTGTC-3′ (105 base-pair (bp)); *RPL13A forward:* 5′-TTGCCTGCCCTTCCTCCATTGTTG-3′, *RPL13A reverse:* 5′-CCTATGTCCCAGGGCTGCCTGT-3′ (90 bp); *EDA forward:* 5′-AGGACTGGCATTCACTGATGTG-3′, *EDA reverse:* 5′-GTCACCCTGTACCTGGAAACTTG-3′ (87 bp); *EDB forward:* 5′-GGTGGACCCCGCTAAACTC-3′, *EDB reverse:* 5′-ACCTTCTCCTGCCGCAACTA-3′ (68 bp); *STAT-1 forward:* 5′-TTCAGGAAGACCCAATCCAG-3′, *STAT-1 reverse:* 5′-TGAATATTCCCCGACTGAGC-3′ (112 bp); *STAT-3 forward:* 5′-AGTGAGTAAGGCTGGGCAGA-3′, *STAT-3 reverse:* 5′-AAGGCACCCACAGAAACAAC-3′ (100 bp). All primers were designed using Primer-Blast (www.ncbi.nlm.nih.gov/tools/primer-blast/) except the EDA and EDB primers [16].

2.6. shRNA knockdown

SMCs were infected with the following validated Mission shRNA pKLO.1 lentiviral particles (Sigma–Aldrich); shSTAT-1 (TRCN0000004265), shSTAT-3 (TRCN0000020842) and non-targeting control shRNA (SHC002V). All shRNAs induce greater than 80% knockdown. SMCs were infected at a multiplicity of infection of 10 in the presence of 8 μg/ml polybrene for 24 h in SMC growth medium. Cultures were then incubated with serum-free DMEM-F12 supplemented with insulin, transferring and selenium (DMEM-F12/ITS) for a further 48 h prior to experimentation. qPCR and Western blotting was used to determine the knockdown efficacy of the STAT-1 and STAT-3 shRNAs (see Supplemental Fig. 2A and B).

2.7. Cell proliferation assay

SMCs were cultured in 96-well plates at 2×10^3 cells per well in SMC growth medium. After 24 h, growth medium was replaced with DMEM-F12/ITS and treated with platelet-derived growth factor-BB (PDGF-BB) with or without OSM (10 ng/ml) for a further 72 h. Cell proliferation was quantified by using CellTitre-Glo luminescent reagent (Promega) according to the instructions of the manufacturer.

2.8. Cell migration analysis

Migration assays were carried out in ChemoTx microtitre plates (Neuro Probe Inc.) as described previously [17]. Briefly, 2×10^4 SMCs (OSM treated or untreated) were placed on 96-well filters. Lower chambers contained 30 μl of serum-free medium containing PDGF-BB (50 ng/ml). The microplate was incubated for 6 h at 37 °C. Filters were stained with Giemsa and SMCs migrating in response to PDGF-BB were quantified by counting each well at three random high power fields. All groups were studied in quadruplicate.