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MCP-induced protein 1 suppresses TNFα-induced VCAM-1 expression in human endothelial cells

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ABSTRACT

Endothelial inflammation plays a critical role in the development and progression of cardiovascular disease, albeit the mechanisms need to be fully elucidated. We here report that treatment of human umbilical vein endothelial cells (HUVECs) with tumor necrosis factor (TNF) α substantially increased the expression of MCP-induced protein 1 (MCPIP1). Overexpression of MCPIP1 protected ECs against TNFα-induced endothelial activation, as characterized by the attenuation in the expression of the adhesion molecule VCAM-1 and monocyte adherence to ECs. Conversely, small interfering RNA-mediated knock down of MCPIP1 increased the expression of VCAM-1 and monocytic adherence to ECs. These studies identified MCPIP1 as a feedback control of cytokines-induced endothelial inflammation.

1. Introduction

A crucial step in atherogenesis is the arterial recruitment of inflammatory cells from the circulation and their transendothelial migration into the subendothelial space of large arteries where they differentiate into macrophages and become functionally active [1,2]. In response to inflammatory stimuli, such as tumor necrosis factor α (TNFα), interleukin 1β (IL)-1β, and interferon γ, endothelial cells (ECs) undergo inflammatory activation, resulting in an increased surface expression of cell adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin, which contributes to the recruitment of inflammatory cells to arterial wall and their transmigration across the wall [3,4]. The activated ECs also secret cytokines and chemokines such as monocyte chemotactic protein 1 (MCP-1), which is a potent inducer for monocyte attachment to ECs and migration into subendothelial space [5,6]. Mice deficient in MCP-1 or its receptor CCR2 had significant reduced atherosclerotic lesions, suggesting that MCP-1/CCR2 interaction has a role in monocyte recruitment in atherosclerosis [7,8].

MCP-induced protein 1 (MCPIP1, also known as ZC3H12A) is a recently identified gene in human peripheral blood monocytes treated with MCP-1 [9,10]. The gene undergoes rapid and potent transcription induction upon stimulation with proinflammatory molecules, such as TNFα, MCP-1, IL-1β and lipopolysaccharide (LPS) [9–13]. Further studies showed that MCPIP1 plays an important role in both physiological and pathological processes related to inflammation [10,11]. In the experiments on cultured cells, MCPIP1 was proved to be a negative regulator of macrophage activation [10]. In a recent report on mice, MCPIP1 deficiency leads to a complex phenotype including severe anemia, autoimmune response and severe inflammatory response and most mice died within 12 weeks of birth [11]. These results suggest that MCPIP1 may critically control inflammation and immunity and would be a potential therapeutic target for treatment of human inflammatory diseases such as atherosclerosis.

Abbreviations: MCPIP1, MCP-induced protein 1; HUVECs, human umbilical vein endothelial cells; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; NF-κB, nuclear factor-κB; EC, endothelial cell; IL-1β, interleukin 1β; MCP-1, monocyte chemotactic protein 1; LPS, lipopolysaccharide

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In this study, we showed that MCPIP1 expression is induced by inflammatory cytokines TNFα and IL-1β and that overexpression of MCPIP1 suppresses cytokine-induced expression of VCAM-1, as well as monocyte adhesion to human ECs. These results indicate that MCPIP1 also negatively regulates proinflammatory activation of vascular ECs.

2. Materials and methods

2.1. Cell culture and reagents

Human umbilical vein endothelial cells (HUVECs) and human CD14+ monocytes were acquired from Lonza Walkersville Inc. (Walkersville, MD) and cultured in EC basal medium-2 and lymphocyte growth media-3 respectively according to the manufacturer instructions. The human acute monocytic leukemia cell line THP-1 was obtained from American Type Culture Collection and was maintained as described previously [10]. Human MCPIP1 expression plasmid was described previously [10]. Human VCAM-1-Luc plasmid was originally generated by Dr. W.C. Aird's Laboratory (Beth Israel Deaconess Medical Center, Boston) and kindly provided by Dr. Mukesh K. Jain (Case Western Reserve University, Cleveland, Ref. [14]). The MCPIP1 rabbit polyclonal antibody was described previously [10]. VCAM-1 (sc-13160) and actin antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PAI-1, iNOS and ICAM-1 antibodies were from Cell Signaling Technology. Anti-VCAM-1-Fluorescein, anti-ICAM-1-Fluorescein and VCAM-1 monoclonal antibody were purchased from R&D Systems, Inc. Human recombinant TNFα, IL-1β and actinomycin D were purchased from Sigma (Saint Louis, MI).

2.2. Immunohistochemistry

Adult, male C57BL/6 wild-type and ApoE$^{-/-}$ mice were fed with high-cholesterol diet for 4 weeks. Arterial segments were isolated and fixed in 4% paraformaldehyde. Paraffin (5 μm) sections were immunostained with rabbit anti human MCPIP1 antibody using the avidin-biotin peroxidase method. This investigation has been approved by the animal use and care committee of Peking University Health Science Center and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

Human atherosclerotic plaque tissue was collected from an anonymous patient with consent as described in an approved human subject protocol from the UCSB Human Subjects Committee in coordination with Cottage Hospital (Study number 06-59) and conforms to the principles outlined in the "Declaration of Helsinki". Human atherosclerotic sections were co-stained with 1:50 diluted rabbit anti human MCIPIP1 antibody (GeneTex, GTX110807) and 1:100 diluted FITC conjugated moue anti-smooth muscle cell (SMC)-Actin antibody (Sigma, F3777) or FITC conjugated anti-CD31, followed by incubation with Alexa fluor 568 conjugated goat anti rabbit IgG antibody (Invitrogen, A11036). Images were taken using Samsun Fluoview confocol system using the no primary slides as negative control.

2.3. Measurement of cell-surface expression of adhesion molecules

The cell-surface expressions of VCAM-1 and ICAM-1 on HUVECs were performed essentially as described [15] but with some modifications using cell ELISA. HUVECs were seeded in 96-well plate and transfected with HA-MCPIP1 or control vector using transPass HUVEC transfection reagent (NEB). 24 h later the cells were stimulated with TNFα for overnight. The stimulated cells were washed with PBS and fixed with 4% (v/v) paraformaldehyde for 30 min at 4 °C. FBS (1% (v/v) in PBS) was used as a blocking reagent. After washing with PBS, cells were incubated with FITC conjugated anti-VCAM-1 or anti-ICAM-1 monoclonal antibody for 1 h at 4 °C. The cell-surface expressions of VCAM-1 and ICAM-1 were measured with a fluorometer (Flx800, BioTek Instrument, Inc.) at 485 nm excitation wavelength and 535 nm emission wavelength.

2.4. Western blot and Q-PCR

HUVECs were transfected with MCPIP1-EGFP or HA-MCPIP1 as well as their control vectors by electroporation using Amaxa Electroporation Unit following the manufacturer's instruction. 24 h later, the transfected cells were exposed to the indicated stimuli. Total protein or RNA was harvested for Western blot or Q-PCR analysis as described previously [10].

2.5. Luciferase assay

HUVECs were seeded into 12-well plate and transfected with Fugene 6 Transfection Reagent (Roche Applied Science) following the manufacturer's instruction. The total amount of plasmid DNA was kept constant within each experiment. Luciferase activity was measured by luciferase Assay System (Promega) and normalized to β-galactosidase activity by cotransfecting the pCMV-βgal plasmid in all experiments. All transfections were performed in triplicate and at least repeated two times.

2.6. mRNA stability assay

HUVECs were transfected with MCPIP1-EGFP or EGFP control vector by electroporation. After 8 h stimulation with TNFα (10 ng/ml), transcription was stopped by adding 5 μg/ml actinomycin D. The cells were harvested and RNA was isolated at different time points as indicated. The mRNA levels of VCAM-1 were measured by Northern blot as described previously [10].

2.7. Short-interfering RNA

The pre-designed siRNA targeting to human MCPIP1 as well as its negative control were purchased from Santa Cruz Biotech. (CA). The siRNA was transfected into HUVECs by electroporation using Amaxa Electroporation Unit following the manufacturer's instruction. 24 h later, the cells were treated with or without TNFα (10 ng/ml) for 8 h. Then the cells were harvested and protein was isolated to assess for MCPIP1 knockdown and VCAM-1 expression.

2.8. Monocyte adhesion assay

THP-1 cells or human CD14+ monocytes were labeled with fluorescein isothiocyanate using a PKH67 fluorescent staining kit (Zynaxis, Inc., Malvern, PA) according to the instructions of the manufacturer. After the HUVECs were stimulated and washed, 2.5×10^5 fluorescein isothiocyanate-labeled THP-1 cells were added to each well and allowed to interact for 60 min at 37 °C. Unbound cells were removed by gently washing with cold PBS. Images were taken using a Nikon Fluorescence Microscopy (Zeiss, Thornwood, NY). Adherent cells were lysed with 50 mmol/l Tris (pH 8.4)/0.1% sodium dodecyl sulfate, and the fluorescence was measured by a fluorometry.

2.9. Statistics

Data were expressed as mean ± S.D. For comparison between two groups, the unpaired Student's test was used. For multiple comparisons, analysis of variance followed by unpaired