Free Radical Biology and Medicine 54 (2013) 125–134

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

NADPH oxidase 4 is required for interleukin-1β-mediated activation of protein kinase Cδ and downstream activation of c-jun N-terminal kinase signaling in smooth muscle

Roman Ginnan, Frances L. Jourd'heuil, Benjamin Guikema, Malorie Simons, Harold A. Singer, David Jourd'heuil *

Center for Cardiovascular Sciences, Albany Medical College, Albany, NY 12208, USA

ARTICLE INFO

Article history:
Received 10 December 2011
Received in revised form
5 September 2012
Accepted 19 September 2012
Available online 26 September 2012

Keywords:
Smooth muscle
NADPH oxidase
Protein kinase C
MAP kinase
JNK
NOX4
NOX1
Interleukin-1β
Reactive oxygen species
iNOS
Free radicals

ABSTRACT

Reactive oxygen species (ROS) are generated in the vascular wall upon stimulation by proinflammatory cytokines and are important mediators of diverse cellular responses that occur as a result of vascular injury. Members of the NADPH oxidase (NOX) family of proteins have been identified in vascular smooth muscle (VSM) cells as important sources of ROS. In this study, we tested the hypothesis that NOX4 is a proximal mediator of IL-1β-dependent activation of PKCδ and increases IL-1β-stimulated c-Jun kinase (JNK) signaling in primary rat aortic VSM cells. We found that stimulation of VSM cells with IL-1β increased PKCδ activity and intracellular ROS generation. SiRNA silencing of NOX4 but not NOX1 ablated the IL-1β-dependent increase in ROS production. Pharmacological inhibition of PKCδ activity as well as siRNA depletion of PKCδ or NOX4 blocked the IL-1β-dependent activation of JNK. Further studies showed that the IL-1β-dependent upregulation of inducible NO synthase expression was inhibited through JNK inhibition and NOX4 silencing. Taken together, these results indicate that IL-1β-dependent activation of PKCδ is modulated by NOX4-derived ROS. Our study positions PKCδ as an important redox-sensitive mediator of IL-1β-dependent signaling and downstream activation of inflammatory mediators in VSM cells.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Vascular diseases such as atherosclerosis, postangioplasty restenosis, posttransplant coronary arteriopathy, and vascular hyporesponsiveness after septic shock all have an inflammatory component associated with their etiologies [1]. In the vessel wall, the inflammatory response leads to the production of cytokines and growth factors, which induce the proliferation and migration of the underlying smooth muscle resulting in thickening of the medial layer, matrix deposition, and formation of a neointima [2]. Mice deficient in the IL-1[1] receptor IL-1R1 present with reduced neointima/media formation after injury [3], whereas animals deficient in the IL-1 receptor antagonist display increased neointimal formation upon injury [4]. IL-1β is expressed by vascular smooth muscle (VSM) cells and other cell types in advanced stages of atherosclerotic plaques as well as in nonatherosclerotic vascular injury models, in which it regulates VSM cell gene expression and proliferation [5–8].

The prototypical signaling pathway associated with IL-1β requires the adaptor protein MyD88 and the recruitment of the IL-1 receptor-associated kinase and TNFα receptor-associated factor 6 (TRAF6) [9,10]. These in turn activate downstream kinases and regulate the transcriptional activation of inflammatory genes. We also identified PLC-γ and PKCδ as important components of IL-1β signaling in cultured rat aortic VSM cells [11]. Most if not all of these signaling elements are sensitive to changes in intracellular redox status, although the significance of these effects has not always been demonstrated in smooth muscle.

A common feature of vascular diseases is the dysregulation of reactive oxygen species (ROS) production in the vascular wall. In this context, some of the most significant sources of ROS are NADPH oxidases (NOXs) because their activation allows for temporal and spatial regulation of intracellular signaling pathways

Abbreviations: DPI, diphenyleneiodonium chloride; Duox, dual oxidase; HE, hydroethidine; IL-1, interleukin-1; JNK, c-Jun kinase; MAPK, mitogen-activated protein kinase; NOX, NADPH oxidase; PKCδ, protein kinase Cδ; PLC-γ, phospholipase C-γ; phox, phagocytic oxidase; ROS, reactive oxygen species; TGF-β, transforming growth factor β; TLR, Toll-like receptor; TRAF6, tumor necrosis factor α receptor-associated factor 6; VSM, vascular smooth muscle

* Corresponding author. Fax.: +518 262 8101.
E-mail address: jourdhd@mail.amc.edu (D. Jourd'heuil).

0891-5849/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2012.09.026

[12]. NOXs are composed of one or two transmembrane proteins (such as NOX, DUOX, and p22phox) and in some cases include regulatory subunits such as p47phox, NOXO1, p40phox, p67phox, or NOXOA1. In MCF-7 cells stimulated with IL-1β, NOX2 is required for the recruitment of TRAF6 to the IL-1 receptor complex [13]. The contribution of NOXs to IL-1β signaling in the vasculature and in VSM cells has not been well characterized although IL-1β-induced ROS production has been previously established in VSM cells [14–16]. In this study, we show that the IL-1β-stimulated production of ROS involves NOX4 and that this results in PKCδ-dependent activation of c-Jun N-terminal kinase (JNK) downstream signaling in rat aortic VSM cells.

Materials and methods

Materials

Antibodies for PKCδ were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), antibody for JNK was from Cell Signaling Technologies (Beverly, MA, USA), and antibodies for inducible nitric oxide synthase (iNOS), NOX4, and β-actin were from Millipore (Billerica, MA, USA), Proteintech (Chicago, IL, USA), and Sigma (St. Louis, MO, USA), respectively. Inhibitors of PKCδ, SP00125 and Protease Inhibitor Cocktail III, were purchased from Calbiochem (La Jolla, CA, USA). All tissue media were purchased from GIBCO–BRL Life Technologies (Carlsbad, CA, USA) unless specifically stated. All other chemicals were purchased from Sigma.

All adenovirus stocks were propagated by the addition of small amounts of virus to human embryonic kidney-293 cells. When cells were ~50% lysed, cells and media were collected, subjected to 3× freeze/thaw cycles, aliquotted, and stored at −80 °C. Titer assays were performed using an adenovirus containing β-galactosidase as a control at matching multiplicity of infection.

Generation of adenoviral small interfering RNA (siRNA) constructs

Adenoviral delivery of siRNA was achieved using the methods developed by Zhao et al. [17]. Target sequences were identified using the Dharmacon Web site [18]. Adenoviral vectors were designed to express siRNA hairpins targeted to rat NOX4 (GenBank Accession No. NM_053524) or rat NOX1 (GenBank Accession No. NM_053683). The target sequences were chemically synthesized as

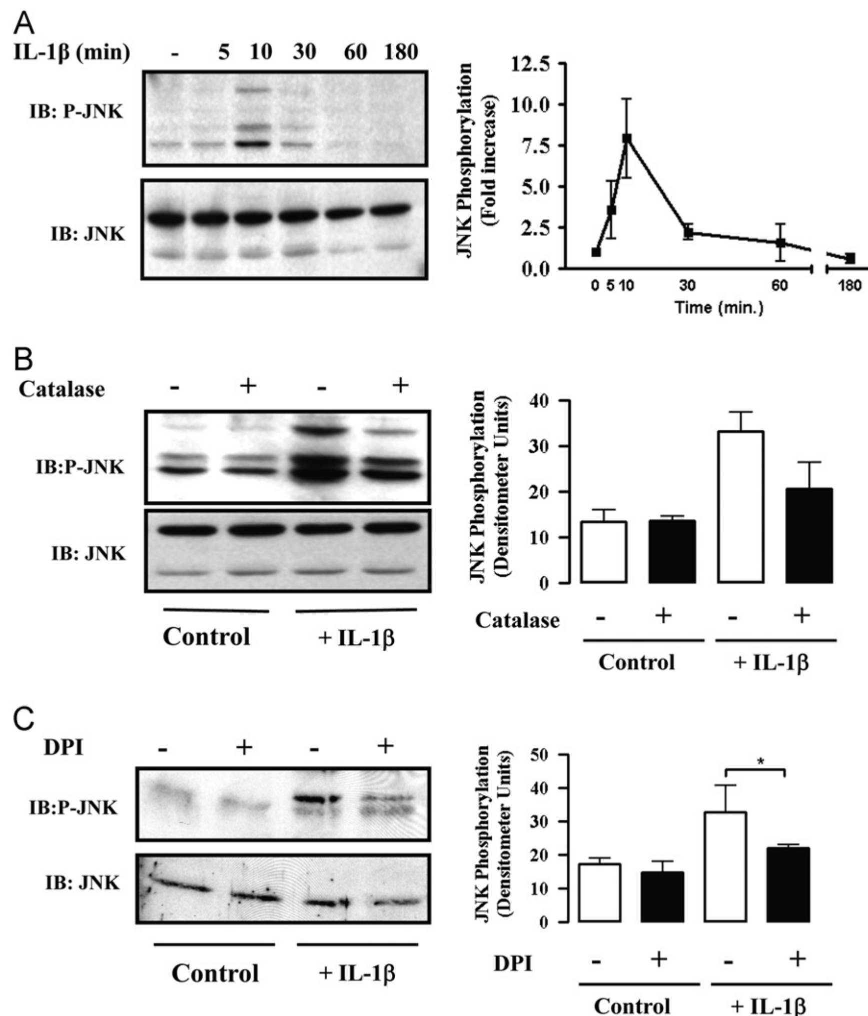

Fig. 1. IL-1β-induced JNK activation is ROS-dependent in rat aortic VSM cells. (A) Time course of IL-1β-induced JNK in VSM cells. VSM cells were stimulated with 10 ng//ml IL-1β for the indicated times. Whole-cell lysates were then resolved using SDS–PAGE and transferred to nitrocellulose membrane as described under Materials and methods. The nitrocellulose membranes were then immunoblotted (IB) with antibody for active c-Jun kinase (P-JNK) and total JNK to ensure equivalent loading of protein in each well. (B) VSM cells were treated with 3000 U/ml catalase 16 h before stimulation with 10 ng//ml IL-1β for 10 min. JNK activation was determined as described for (A). (C) VSM cells were treated with 10 μM DPI for 30 min before stimulation with 10 ng//ml IL-1β for 10 min. JNK activation was determined as described for (A). On the right of each immunoblot is the quantitation of three or four separate experiments.

Download English Version:

https://daneshyari.com/en/article/1908704

Download Persian Version:

https://daneshyari.com/article/1908704

Daneshyari.com