Available online at www.sciencedirect.com
ScienceDirect
Free Radical Biology & Medicine 42 (2007) 343–352
www.elsevier.com/locate/freeradbiomed

Original Contribution

Discoidin domain receptor 1 mediates collagen-induced nitric oxide production in J774A.1 murine macrophages

Sang-Hyun Kim [a,*], Soyoung Lee [a], Kyoungho Suk [a], Hyun Bark [b], Chang-Duk Jun [c], Dae-Ki Kim [d], Cheol-Hee Choi [b], Teizo Yoshimura [e]

[a] Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu 700-422, Korea
[b] Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501-759, Korea
[c] Department of Life Science, Gwangju Institutive of Science and Technology, Gwangju 500-712, Korea
[d] Department of Immunology, Chonbuk National University Medical School, Jeonju 561-108, Korea
[e] Laboratory of Molecular Immunoregulation, National Cancer Institute at Frederick, Frederick, MD 21702, USA

Received 13 May 2006; revised 8 September 2006; accepted 28 October 2006
Available online 3 November 2006

Abstract

Nitric oxide (NO) is an important regulator of immune responses. Effects of cytokines, such as tumor necrosis factor (TNF)-α or IFN-γ, and bacterial products, such as lipopolysaccharide, on macrophage NO production have been well documented; however, the role of the extracellular matrix proteins, including collagen, in this process remains unclear. We previously reported that discoidin domain receptor 1 (DDR1), a nonintegrin collagen receptor, was expressed in human macrophages, and its activation facilitated their differentiation as well as cytokine/chemokine production. Here, we examined the role for DDR1 in collagen-induced NO production using the murine macrophage cell line J774 cells that endogenously express DDR1. Activation of J774 cells with collagen induced the expression of inducible NO synthase (iNOS) and NO production. Inhibition of DDR1, but not β1-integrins, abolished collagen-induced iNOS and NO production. Activation of J774 cells with collagen-activated nuclear factor-κB, p38 mitogen-activated protein kinase (MAPK), and c-jun N-terminal kinase (JNK) and a pharmacological inhibitor of each signaling molecule significantly reduced collagen-induced NO production. Thus, we have demonstrated, for the first time, that the interaction of DDR1 with collagen induces iNOS expression and subsequent NO synthesis in J774 cells through activation of NF-κB, p38 MAPK, and JNK and suggest that intervention of DDR1 signaling in macrophages may be useful in controlling inflammatory diseases in which NO plays a critical role.

Keywords: Discoidin domain receptor 1; Nitric oxide; Macrophages; Nuclear factor-κB; p38 kinase; c-jun N-terminal kinase

Introduction

Macrophages contribute to the development of inflammatory responses by secreting an array of cytokines and chemokines in a tissue microenvironment. Proinflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, are potent activators of macrophages and up-regulate the expression and production of cytokines and chemokines. At inflammatory sites, they also interact with the components of the extracellular matrix (ECM) through receptors, among which integrins are the best known, and are activated for increased release or production of cytokines/chemokines. For instance, activation of monocytes with collagen, the most abundant protein in the ECM, induced IL-1 release. Collagen-induced IL-1 release was only partially inhibited by an antibody (Ab) against α2β1-integrin, a classic cell-surface collagen receptor [1], suggesting that the presence of an alternative receptor(s) is involved in monocytes/macrophage–collagen interaction.

Abbreviations: DDR1, discoidin domain receptor 1; iNOS, inducible NO synthase; JNK, c-jun N-terminal kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; ECM, extracellular matrix; TNF, tumor necrosis factor; IL, interleukin; NF-κB, nuclear factor-κB; LPS, lipopolysaccharide; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PDTC, pyrrolidine dithiocarbamate; PGA, recombinant protein G-agarose; RT-PCR, reverse transcriptase-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

* Corresponding author. Fax: +82 53 423 4838.
E-mail address: shkim72@knu.ac.kr (S.-H. Kim).

0891-5849/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2006.10.052

Nitric oxide (NO) regulates many physiological and pathophysiological processes as well as the maintenance of neuronal communication, vascular regulation, and immune system. Stimulants, such as LPS, cytokines, ECM proteins, induce macrophages to express inducible NO synthase (iNOS) that catalyzes the production of NO [2,3]. This macrophage-derived NO is an important component of host defense against pathogens and tumor cells [4–6]. Previously, the role of collagen in NO production was reported [3]. Stimulation of macrophages with collagen induced iNOS expression and NO production; however, the exact mechanisms of collagen-induced iNOS expression remain unidentified.

Discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase (RTK) with a unique extracellular domain homologous to discoidin 1 of *Dictyostelium discoideum* [7,8]. Unlike other RTKs which are activated by growth factors, DDR1 is activated by the binding to its ligand collagen. DDR1 is constitutively expressed in epithelial cells of normal tissues, such as lung, kidney, colon, and brain, and also in tumor cells of epithelial origin, such as mammary, ovarian, and lung carcinomas [8–10]. Primary vascular smooth muscle cells isolated from DDR1-null mice showed decreased proliferation, collagen attachment, and migration in vitro [11,12].

We previously reported that the expression of DDR1 could be induced in leukocytes, including neutrophils, monocytes, and lymphocytes, in vitro. In vivo, tissue-infiltrating mononuclear cells, especially macrophages, were positive for DDR1 mRNA. Activation of DDR1 with collagen facilitated the differentiation and chemokine production of macrophages, and maturation of monocyte-derived dendritic cells via activation of downstream signaling pathways involving nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) [13–15]. These previous results led us to hypothesize that DDR1 may mediate collagen-induced NO synthesis in macrophages.

In the present study, we tested our hypothesis using the murine macrophage cell line J774 and demonstrate that activation of DDR1 with collagen up-regulates iNOS expression and concomitant NO production in these cells by a mechanism dependent on NF-κB, p38 MAPK, and JNK. Thus, our study provides a mechanism for previously unidentified collagen-induced NO synthesis in macrophages and DDR1 is likely to contribute to the development of inflammatory responses in a tissue microenvironment.

Materials and methods

Reagents

A soluble form of bovine collagen type I [15] was purchased from Sigma (St. Louis, MO). Although there was no detectable level of endotoxin in the preparation (less than 10 pg/ml) by a colorimetric Limulus ameobocyte lysate assay (Whittaker Bioproducts, Walkersville, MD), it was treated on polymyxin B-agarose (Sigma) to further rule out endotoxin contamination. LPS (*Escherichia coli* 026:B6) was also purchased from Sigma. SB203580, PD98059, SP600125, and pyrrolidine dithiocarbamate (PDTC) were from Calbiochem (La Jolla, CA). Rabbit polyclonal Abs against DDR1 (C-20), iNOS, β1-integrin, IκBα, and p65 NF-κB were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse mAbs against phosphotyrosine (4G10) and anti-β1-integrin-blocking Ab (DE9) were from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal Abs against phosphorylated or nonphosphorylated p38, JNK, and ERK were from Cell Signaling Technology (Beverly, MA). An anti-human IgG1-Fc mAb, DMEM, recombinant protein G-agarose (PGA), and TRIzol reagent were from Invitrogen (Gaithersburg, MD).

Cell cultures

The BALB/c murine macrophage cell line J774A.1 (ATCC TIB-67) was grown in DMEM supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% non-heat-inactivated fetal bovine serum in 5% CO_2 at 37°C. Thioglycollate (TG)-elicited macrophages were harvested 3 days after ip injection of 2.5 ml TG to 8-week-old BALB/c mice and isolated, as previously reported [16]. Peritoneal lavage was performed by using 8 ml of HBSS, which contained heparin. Then, cells were distributed in culture media.

Western blot analysis

Cells were plated at 1×10^6 cells/ml and incubated with collagen for indicated times. After incubation, they were rinsed twice with ice-cold phosphate-buffered saline (PBS) and then lysed in ice-cold lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 50 mM Hepes, 1% Triton-X, 10% glycerol, 1 mM sodium fluoride, 1 mM EDTA, 2 mM sodium orthovanadate, 1 mg/ml leupeptin, 1 mM PMSF, 1 mg/ml aprotinin). The lysates were spun in a microcentrifuge for 20 min at 4°C, and the supernatants were collected. The samples were incubated with an approximately 20 μl packed volume of PGA for 1 h at 4°C. After brief centrifugation, supernatants were collected, mixed with 1 μg/ml polyclonal anti-DDR1 IgG, and incubated for 1 h at 4°C. Twenty microliters of PGA was then added and incubated for another 1 h. IgG-coupled PGA was washed three times with washing buffer (50 mM Hepes, 150 mM NaCl, 0.1% Triton X-100, and 10% glycerol), and 20 μl double-strength sample buffer (20% glycerol, 6% SDS, 10% 2-mercaptoethanol) was added. The samples were boiled for 10 min. Eluted proteins were analyzed on 8% polyacrylamide gels by SDS-PAGE and transferred electrophoretically to nitrocellulose membranes at 150 mA for 1 h in a semidry system. The membranes were incubated with either anti-DDR1 IgG, or anti-phosphotyrosine IgG, followed by an appropriate secondary Ab coupled with horseradish peroxidase. Peroxidase activity was visualized by the Enhanced Chemiluminescence Detection System (Amersham Pharmacia Biotech, Piscataway, NJ). To detect iNOS, IκBα, NF-κB (p65), p38 MAPK, JNK, or ERK, each cell lysate was directly mixed with 20 μl of sample buffer and loaded on 8–12% polyacrylamide gels.