Negative regulation of JNK-mediated monocyte adhesion to human pulmonary microvascular endothelial cells by p38 MAPK

Tokiko Suzuki, Sailesh Palikhe, Kimimasa Sakata, Natsumi Mizuno, Yuichi Hattori

Department of Molecular and Medical Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

[Background] Endothelial cell injury is an important component in the pathophysiology of acute lung injury. By a variety of stimuli, circulating leukocytes adhere to vascular endothelial cells and transmigrate to the interstitium. Adhesion molecules play a pivotal role in leukocyte-endothelial adhesion. The mitogen-activated protein kinase (MAPK) signaling cascades are generally thought to be involved in the pathogenesis of acute lung injury, but their roles in regulating leukocyte adhesion to pulmonary microvascular endothelial cells is poorly understood. In the present study, we examined the involvement of the MAPK family member in monocyte adhesion to human pulmonary microvascular endothelial cells (HPMVECs) stimulated with lipopolysaccharide (LPS) and interferon-γ (IFN-γ).

[Methods] mRNA expression and protein expression in cells were analyzed by quantitative RT-PCR and immunoblotting, respectively. Secreted IL-6 in media was evaluated by ELISA assay. Cellular localization of TLR-4 was examined by immunofluorescence. The monocytic cell line THP-1 adhesion to endothelial cells was evaluated by microscopic observation.

[Results] HPMVECs exhibited the upregulation of surface expression of TLR-4 by IFN-γ, resulting in potentiation of inflammatory cytokine release by LPS stimulation. All MAPKs, ERK1/2, JNK, and p38, were activated by simultaneous stimulation with LPS/IFN-γ. The mRNA and protein expression levels of the adhesion molecule ICAM-1 were eliminated by the JNK inhibitor, suggesting that ICAM-1 expression is positively regulated by JNK. The p38 inhibitor significantly enhanced ICAM-1 expression. JNK activation in cells stimulated with LPS/IFN-γ was potentiated by p38 inhibitors SB203580 and RWJ67657. These suggest the negative regulation of JNK activation by p38 in HPMVECs. ERK1/2 activation was not responsible for the LPS/IFN-γ-induced ICAM-1 upregulation in HPMVECs. THP-1 monocyte adhesion to HPMVECs under LPS/IFN-γ stimulation was inhibited by the JNK inhibitor and enhanced by the p38 inhibitor.

[Conclusion] In HPMVECs stimulated with LPS/IFN-γ, JNK mediates ICAM-1 expression that can facilitate leukocyte adherence and transmigration, while p38 MAPK was found to negatively regulate the upregulation of ICAM-1 through inhibition of JNK activation.