The Role of Human Antigen R, an RNA-binding Protein, in Mediating the Stabilization of Toll-Like Receptor 4 mRNA Induced by Endotoxin: A Novel Mechanism Involved in Vascular Inflammation.

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摘要

Abstract

OBJECTIVE: Lipopolysaccharide (LPS) interacts with toll-like receptor 4 (TLR4) and induces proliferation of vascular smooth muscle cells (VSMCs) which plays a causal role in atherogenesis. The role of TLR4 expression and regulation in LPS-stimulated VSMCs remains unclear. TLR4 mRNAs often contain AU-rich elements (AREs) in their 3' untranslated regions (3'UTR) which have a high affinity for RNA-binding proteins. It is not know whether the RNA-binding protein, human antigen R (HuR), regulates TLR4 expression in human aortic smooth muscle cells (HASMCs). METHODS AND RESULTS: Stimulation of HASMCs with LPS significantly increased the cytosolic HuR level in vitro. Immunoprecipitation and RT-PCR demonstrated that LPS markedly increased the interaction of HuR and 3'UTR of TLR4 mRNA. The reporter plasmid, which contains the 3'UTR of TLR4 mRNA, significantly increased luciferase reporter gene expression in LPS-induced HASMCs. These data suggest that the 3'UTR of TLR4 mRNA confers LPS responsiveness and that HuR modulates 3'UTR-mediated gene expression. Knock-down of HuR inhibited LPS-induced TLR4 mRNA stability in HASMCs and luciferase reporter gene expression in CMV-Luciferase-TLR4 3'UTR-transfected HASMCs. In addition, inhibition of NADPH oxidase activity by diphenylene

iodonium, knock-down of Rac1 gene expression by siRNA, and decrease of p38 MAPK activity by SB203580 significantly decreased the cytosolic HuR level, which mediates TLR4 mRNA stability. CONCLUSIONS: Activation of NADPH oxidase and the MAPK-signaling pathway contribute to HuR-mediated stabilization of TLR4 mRNA induced by LPS in HASMCs. In the balloon injured rabbit aorta model, systemic inflammation induced by LPS caused intimal hyperplasia and increased TLR4 and HuR expression..