Nitric Oxide Modulates Pro- and Anti-inflammatory Cytokines in Lipopolysaccharide-Activated Macrophages

何爲斌

Wu CH;Chen TL;Chen TG;Ho WP;Chiu WT;Chen RM

摘要

Abstract

Background: Sepsis is a serious and life-threatening syndrome that occurs in intensive care unit patients. Lipopolysaccharide (LPS) has been implicated as one of major causes of sepsis. Nitric oxide (NO) and cytokines are involved in sepsis-induced inflammatory responses. This study is aimed at evaluating the effects of NO on the modulation of pro- and anti-inflammatory cytokines in LPS-activated macrophages and its possible mechanism. Methods: N-Monomethyl arginine (NMMA), an inhibitor of NO synthase, was used in this study to suppress NO production. Mouse macrophage-like Raw 264.7 cells were exposed to LPS, NMMA, or a combination of NMMA and LPS. Cell viability was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The amounts of nitrite, an oxidative product of NO, in the culture medium were quantified according to the Griess reaction method. Enzyme-linked immunosorbent assay and reverse-transcriptase polymerase chain reaction were carried out to determine the expression of tumor necrosis factor (TNF)-a, interleukin (IL)-1 β, and IL-10 in macrophages. Results: Exposure of macrophages to LPS, NMMA, and a combination of NMMA and LPS for 24 hours did not affect cell viability. LPS significantly increased the amounts of nitrite in macrophages ($p < 0.01$). Treatment with NMMA decreased LPS-enhanced nitrite ($p < 0.01$) in a concentration-dependent manner. Analyses of enzyme-linked immunosorbent assays and reverse-transcriptase polymerase chain reaction revealed that LPS significantly induced TNF-a, IL-1 β, and IL-10 proteins and mRNA ($p < 0.01$). A combined treatment with NMMA and LPS significantly blocked LPS-induced TNF-a and IL-1 β ($p < 0.01$), but synergistically enhanced LPS-induced IL-10 ($p < 0.05$) protein and RNA. Conclusion: This study has shown that NO suppression can inhibit LPS-induced TNF-a and IL-1 β but enhance IL-10, and the modulation occurs at a

pretranslational level.