CheNitric oxide modulates pro-and anti-inflammatory cytokines in lipopolysaccharide-activated macrophages.

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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-di-phenyltetrazolium 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)-alpha, interleukin (IL)-1 beta, 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-alpha, IL-1 beta, and IL-10 proteins and mRNA ($p < 0.01$). A combined treatment with NMMA and LPS significantly blocked LPS-induced TNF-alpha and IL-1 beta ($p < 0.01$), 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-alpha and IL-1 beta but enhance IL-10, and the modulation occurs at a pretranslational level.