Induction of Nitric Oxide Synthease in RAW 264.7 Macrophages by Lipoteichoic Acid from Staphylococcus Aureus: Involvement of Protein Kinase C- and Nuclear Factor-kB-Dependent Mechanisms.

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Abstract

This study investigates the signaling pathway involved in inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) release caused by Staphylococcus aureus lipoteichoic acid (LTA) in RAW 264.7 macrophages. A phosphatidylcholine-phospholipase C (PC-PLC) inhibitor (D-609) and a phosphatidylinositol-phospholipase C (PI-PLC) inhibitor (U-73122) attenuated LTA-induced iNOS expression and NO release. Two PKC inhibitors (Go 6976 and Ro 31-8220), an NF-kappaB inhibitor (pyrrolidine dithiocarbamate; PDTC), and long-term (24 h) 12-phorbol-13-myristate acetate (PMA) treatment each also inhibited LTA-induced iNOS expression and NO release. Treatment of cells with LTA caused an increase in PKC activity; this stimulatory effect was inhibited by D-609, U-73122, or Ro 31-8220. Stimulation of cells with LTA caused IkappaB-alpha phosphorylation and IkappaB-alpha degradation in the cytosol, and translocation of p65 and p50 NF-kappaB from the cytosol to the nucleus. Treatment of cells with LTA caused NF-kappaB activation by detecting the formation of NF-kappaB-specific DNA-protein complexes in the nucleus; this effect was inhibited by Go 6976, Ro 31-8220, long-term PMA treatment, PDTC, L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK), and calpain inhibitor I. These results suggest that LTA might activate PC-PLC and PI-PLC to induce PKC activation, which in turn initiates NF-kappaB activation, and finally induces iNOS expression and NO release in RAW 264.7 macrophages.