Peptidoglycan-Induced IL-6 Production in RAW 264.7 Macrophages Is Mediated by Cyclooxygenase-2, PGE2/PGE4 Receptors, Protein Kinase A, I kappaB Kinase, and NF- kappaB.

許銘仁

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摘要

Abstract

In this study, we investigated the signaling pathway involved in IL-6 production caused by peptidoglycan (PGN), a cell wall component of the Gram-positive bacterium, Staphylococcus aureus, in RAW 264.7 macrophages. PGN caused concentration- and time-dependent increases in IL-6, PGE(2), and cAMP production. PGN-mediated IL-6 production was inhibited by a nonselective cyclooxygenase (COX) inhibitor (indomethacin), a selective COX-2 inhibitor (NS398), a PGE(2) (EP2) antagonist (AH6809), a PGE(4) (EP4) antagonist (AH23848), and a protein kinase A (PKA) inhibitor (KT5720), but not by a nonselective NO synthase inhibitor (N(G)-nitro-l-arginine methyl ester). Furthermore, PGE(2), an EP2 agonist (butaprost), an EP2/PGE(3) (EP3)/EP4 agonist (misoprostol), and misoprostol in the presence of AH6809 all induced IL-6 production, whereas an EP1/EP3 agonist (sulprostone) did not. PGN caused time-dependent activations of IkappaB kinase alphabeta (IKKdbeta) and p65 phosphorylation at Ser(276), and these effects were inhibited by NS398 and KT5720. Both PGE(2) and 8-bromo-cAMP also caused IKKdbeta kinase alphabeta phosphorylation. PGN resulted in two waves of the formation of NF-kappaB-specific DNA-protein complexes. The first wave of NF-kappaB activation occurred at 10-60 min of treatment, whereas the later wave occurred at 2-12 h of treatment. The PGN-induced increase in kappaB luciferase activity was inhibited by NS398, AH6809, AH23848, KT5720, a protein kinase C inhibitor (Ro31-8220), and a p38 MAPK inhibitor (SB203580). These results suggest that PGN-induced IL-6 production involves COX-2-generated PGE(2), activation of the EP2 and EP4 receptors, cAMP formation, and the activation of PKA, protein kinase C, p38

MAPK, IKKdbeta, kinase alphabeta, p65 phosphorylation, and NF-kappaB. However, PGN-induced NO release is not involved in the signaling pathway of PGN-induced IL-6 production.