Peptidoglycan Induces Nuclear Factor-kB Activation and Cyclooxygenase-2 Expression via Ras, Raf-1, and ERK in RAW 264.7 Macrophages

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摘要

Abstract

In this study, we investigated the signaling pathway involved in cyclooxygenase-2 (COX-2) expression caused by peptidoglycan (PGN), a cell wall component of the Gram- positive bacterium Staphylococcus aureus, in RAW 264.7 macrophages. PGN caused dose- and time-dependent increases in COX-2 expression, which was attenuated by a Ras inhibitor (manumycin A), a Raf-1 inhibitor (GW 5074), and an MEK inhibitor (PD 098059). Treatment of RAW 264.7 macrophages with PGN caused time-dependent activations of Ras , Raf-1, and ERK. The PGN-induced increase in Ras activity was inhibited by manumycin A. Raf-1 phosphorylation at Ser-338 by PGN was inhibited by manumycin A and GW 5074. The PGN- induced increase in ERK activity was inhibited by manumycin A, GW 5074, and PD 098059. Stimulation of cells with PGN activated IkappaB kinase alpha/beta (IKalpha/beta), IkappaBalpha phosphorylation, IkappaBalpha degradation, and kappaB-luciferase activity. Treatment of macrophages with an NF-kappaB inhibitor (pyrrolidine dithiocarbamate), an IkappaBalpha phosphorylation inhibitor (Bay 117082), and IkappaB protease inhibitors (L-1-tosylamido-2-phenylethyl chloromethyl ketone and calpain inhibitor I) all inhibited PGN-induced COX-2 expression. The PGN-mediated increase in the activities of IKKalpha/beta and kappaB-luciferase were also inhibited by the Ras dominant negative mutant (RasN17), manumycin A, GW 5074, and PD 098059. Further studies revealed that PGN induced the recruitment of p85alpha and

Ras to Toll-like receptor 2 in a time-dependent manner. Our data demonstrate for the first time that PGN activates the Ras/Raf-1/ERK pathway, which in turn initiates IKKalpha/beta and NF-kappaB activation, and ultimately induces COX-2 expression in RAW 264.7 macrophages.