25. THE ROLE OF NFκB IN (1→3)-β-GLUCAN (ZYMOSAN A) INDUCED TNF-α PRODUCTION — S. Young[1], J. Ye[2], D.G. Frazer[1], X. Shi[2] and V. Castranova[2, 1] Engineering Control and Technology Branch, and [2] Pathology and Physiology Research Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV

The signal transduction pathway in the inflammatory response caused by a fungal cell wall component (1\3)-β-glucan is not well understood. The present study used zymosan A-induced tumor necrosis factor (TNF)-α production as a model to explore the signal transduction pathway for β-glucan stimulation in RAW264.7 cells. Zymosan A increased TNF-α production in RAW264.7 cells in a time and concentration dependent pattern with the optimal time and concentration occurring at 23 hr and 100 μg/mL zymosan A, respectively. A gel shift assay was used to examine the DNA-binding activity of NFκB in the zymosan A- treated cells. This NFκB activity was enhanced by this stimulant. NFκB activation was associated with TNF-α production, since the cells pre-treated with a known NFκB inhibitor (caffeic acid phenethyl ester) decreased both NFκB activation and TNF-α production. The dependence of TNF-α transcriptional initiation on κB sites was investigated using a luciferase reporter assay. Both wild type and κB-mutated type TNF-α promoters were utilized to study the dependence of TNF-α promoter on κB sites. The results demonstrated that the activation of TNF-α promoter was dependent on the activation of NFκB. A NFκB specific reporter was used in conjunction with TNF-α wild type reporter to evaluate the temporal relationship between the NFκB activation and induction of TNF-α reporter activity. The results showed that the peak of NFκB activation occurred at 5 hrs which proceeded the peak of TNF-α promoter activation (30 hrs). The above results suggest that activation of NFκB is one of the pathways involved in zymosan A-induced TNF-α production.

ABSTRACTS

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