Roles of p38MAPK, PKC and PI 3-K in the signaling pathways of NADPH oxidase activation and phagocytosis in bovine polymorphonuclear leukocytes

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Polymorphonuclear leukocytes (PMN) play a central role at the early stage of non-specific host defense against invading pathogens. Although the bactericidal response of PMN is essential for the host system, the signaling pathways involved in it have not been fully elucidated. In this study, using isolated bovine PMN, the effects of three kinase inhibitors, p38 MAPK, PKC and PI 3-K, on the activation of NADPH oxidase and phagocytosis were examined to clarify the signaling pathways in these PMN functions.

The $^{\cdot}O_2^-$ production from NADPH oxidase in PMN stimulated with serum-opsonized zymosan (OZ) was assessed by a method combining ESR and spin-trapping with 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) and luminol-dependent chemiluminescence spectrometry. In OZ-stimulated PMN, the significant increase of the $^{\cdot}O_2^-$ production was observed and three inhibitors, SB203580 for p38 MAPK, GF109203X for PKC and wortmannin for PI 3-K, caused the significant attenuation of this increased-response. Since the phosphorylation of p47 phox, a cytosolic component of NADPH oxidase, was known to be a trigger of the activation of NADPH oxidase, the in vitro p47 phox phosphorylation was assayed. The p47 phox phosphorylation was induced by OZ-stimulation and three inhibitors suppressed its phosphorylation. In the case of phagocytosis of PMN, the amount of fluorescent latex particles ingested by PMN was measured by flow cytometry. Phagocytic activity of PMN was inhibited by SB203580 and wortmannin but not GF109203X. Furthermore, OZ-induced formation of F-actin, which is essential for the phagocytosis, in PMN was also inhibited by SB203580 and wortmannin but not GF109203X. These results indicated that (1) all kinases examined, p38 MAPK, PKC and PI 3-K, were required for the activation of NADPH oxidase through phosphorylation of p47 phox, and (2) p38MAPK and PI 3-K were required for the activation of phagocytic activity of PMN but PKC was not. From the present study, it was suggested the different roles of these kinases in the signaling pathways in NADPH oxidase activation and phagocytosis with F-actin formation of PMN.