

# **Involvement of protein kinases in the potentiation of lipopolysaccharide-induced inflammatory mediator formation by thapsigargin in peritoneal macrophages.**

陳炳常

Chen BC;Hsieh SL and Lin WW

摘要

## **Abstract**

We have explored the regulatory roles played by  $Ca^{2+}$ -dependent signaling on lipopolysaccharide (LPS)-induced nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-alpha), and interleukin-6 (IL-6) release in mouse peritoneal macrophages. To elevate intracellular  $Ca^{2+}$ , we used thapsigargin (TG) and UTP. Although LPS alone cannot stimulate NO synthesis, co-addition with TG, which sustainably increased  $[Ca^{2+}]_i$ , resulted in NO release. UTP, via acting on P2Y6 receptors, can stimulate phosphoinositide (PI) turnover and transient  $[Ca^{2+}]_i$  increase, however, it did not possess the NO priming effect. LPS alone triggered the release of PGE2, TNF-alpha, and IL-6; all of which were potentiated by the presence of TG, but not of UTP. The stimulatory effect of LPS plus TG on NO release was inhibited by the presence of Ro 31-8220, Go6976, KN-93, PD 098059, or SB 203580, and abolished by BAPTA/AM and nuclear factor kappaB (NF-kappaB) inhibitor, PDTC. PGE2, TNF-alpha, and IL-6 release by LPS alone were attenuated by Ro 31-8220, Go6976, PD 098059, SB 203580, and PDTC. Using L-NAME, soluble TNF-alpha receptor, IL-6 antibody, NS-398, and indomethacin, we performed experiments to understand the cross-regulation by the four mediators. The results revealed that TNF-alpha up-regulated NO, PGE2, and IL-6 synthesis; PGE2 up-regulated NO, but down-regulated TNF-alpha synthesis; and PGE2 and IL-6 mutually up-regulated reciprocally. Taken together, murine peritoneal macrophages required a sustained  $[Ca^{2+}]_i$  increase, which proceeds after TG, but not UTP, stimulation, to enhance LPS-mediated release of inflammatory mediators, particularly for NO induction.

Activation of PKC-, ERK-, and p38 MAPK-dependent signaling also are essential for LPS action. The positive regulatory interactions among these mediators might amplify the inflammatory response caused by endotoxin.