

## Mitogen-Activated Protein Kinases 在 Lipoteichoic Acid 引發巨噬細胞

### 一氧化氮合成酵素表現之訊號

## Studies on the Role of Mitogen-Activated Protein Kinases in Lipoteichoic Acid-Mediated Nitric Oxide Synthase Expression in RAW 264.7 Macrophages

### 中文摘要

本論文主要在探討 mitogen-activated protein kinases (MAPKs) 路徑在 lipoteichoic acid (LTA) 刺激 RAW264.7 巨噬細胞 iNOS 表現及 NO 釋放所扮演的角色。先前的實驗已證實 LTA 以時間及劑量相關的方式刺激 iNOS 表現及 NO 釋放。而我們發現，phosphatidylinositol 3-kinase (PI3K) 抑制劑 wortamanin 及 LY294002 以劑量相關方式抑制 LTA 所引發 iNOS 表現及 NO 釋放。而 MEK 抑制劑 PD 98059 及 p38 抑制劑 SB 203580 也以劑量相關的方式抑制 LTA 所引發之 iNOS 表現及 NO 釋放；然而 Ras 活化的抑制劑 FPT inhibitor 對 LTA 引發之反應則沒有任何影響。LTA 以時間及劑量相關的方式引發 p44/42 MAPK 之活化，當加入 tyrosine kinase 抑制劑 (tyrphostin AG126、genistein)、PKC 抑制劑 (Go 6976、Ro 31-8220)、wortamanin、PD 98059、SB 203580 及 phorbol 12-myristate 13-acetate (PMA) 長時間 (24 h) 處理，發現 tyrphostin AG126、genistein 及 PD 98059 會抑制 LTA 引發 p44/42 MAPK 之活化，而 wortamanin、Go 6976、Ro 31-8220、SB 203580 及 PMA 長時間處理則不影響 LTA 的作用，表示 LTA 所引發之 p44/42 MAPK 的活化可受到上游 tyrosine kinase 之調控，但並不會受到 PI3K、PKC 及 p38 MAPK 的調控。LTA 也可以時間相關的方式引發 p38 MAPK 活性的增加，當加入 wortamanin、LY294002、tyrphostin AG126、genistein、Go 6976、Ro 31-8220 或 SB 203580 等抑制劑及 PMA 長時間處理，這些抑制劑及 PMA 長時間處理皆有抑制作用，表示 LTA 刺激 p38 MAPK 的活性增加可受到上游 PI3K、tyrosine kinase 及 PKC 之調控。NF- $\kappa$ B 抑制劑 pyrrolidine dithiocarbamate (PDTTC) 與 I $\kappa$ B protease 抑制劑 L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) 皆可抑制 LTA 所引發 iNOS 表現及 NO 釋放。由 Electrophoretic mobility shift assay (EMSA) 的結果發現 LTA 可使 NF- $\kappa$ B 的與 DNA 結合的作用增加，於 30 分鐘時達最大反應，但 120 分鐘後反應明顯地減少，當加入 wortamanin、LY294002、tyrphostin AG126、genistein、PD 98059、SB 203580、PDTTC 與 TPCK，發現這些抑制劑皆可抑制 LTA 的作用。綜合以上的結果得知，在 RAW264.7 巨噬細胞中，LTA 可經由 p44/42 及 p38 MAPK 二條訊號傳遞路徑活化 NF- $\kappa$ B，而進一步調控 iNOS 表現及 NO 釋放。而 p38 MAPK 可受到上游 PI3K、tyrosine kinase 及 PKC 之調控。而 p44/42 MAPK 之活化僅受到上游 tyrosine kinase 之調控，但並不會受到 PI3K 及 PKC 之調控。

## 英文摘要

Our previous study has shown that lipoteichoic acid (LTA) caused time- and concentration-dependent increases of inducible nitric oxide synthase (iNOS) expression and NO release. In this study, we investigated the role of mitogen-activated protein kinases (MAPKs) on LTA-mediated iNOS expression and NO release in RAW264.7 macrophages. The phosphatidylinositol 3-kinase (PI3K) inhibitors (wortamanin and LY294002) inhibited the LTA-induced iNOS expression and NO release in a concentration-dependent manner. The LTA-induced increases in iNOS expression and NO release were also attenuated by the MEK inhibitor (PD 98059) and p38 MAPK inhibitor (SB 203580), but not by FPK inhibitor II. Treatment of RAW 264.7 macrophages with LTA caused time- and concentration-dependent activations of p44/42 MAPK. Moreover, the LTA-induced p44/42 MAPK activation was inhibited by the tyrosine kinase inhibitors (tyrphostin AG126 and genistein) and PD 98059, but not wortamanin, the PKC inhibitors (Go 6976 and Ro 31-8220), long term PMA (24 h) treatment, or SB 203580. These results suggested that LTA-mediated activation of p44/42 MAPK was regulated by upstream tyrosine kinase, but not by PI3K, PKC and p38 MAPK. Stimulation of RAW 264.7 macrophages with LTA also induced p38 MAPK activation; this effect was inhibited by wortamanin, LY294002, tyrphostin AG126, genistein, Go 6976, Ro 31-8220 and long term PMA treatment, indicating that LTA might act through the pathways of PI3K, tyrosine kinase and PKC to induce p38 MAPK activation. The NF- $\kappa$ B inhibitor (PDTC) and I $\kappa$ B protease inhibitor (TPCK) reduced concentration-dependently the LTA-induced NO release and iNOS expression. Treatment of RAW 264.7 macrophages with LTA stimulated NF- $\kappa$ B specific DNA-protein complex formation in nuclear extracts; this effect was inhibited by wortamanin, LY294002, genistein, tyrphostin AG126, PD 98059, SB 203580, PDTC and TPCK. Taken together, these results indicated that in RAW264.7 macrophages, LTA might activate p44/42 and p38 MAPK, which in turn resulting in stimulation NF- $\kappa$ B DNA-protein binding, and finally initiated iNOS expression and NO release. Both events required the activation of an upstream protein tyrosine kinase. The activation of p38 MAPK was downstream signal of LTA-mediated activation of PI3K and PKC, whereas that of p44/42 MAPK was PI3K- and PKC-independent.