Ras/PI3K/Akt 及 p38 MAPK/MSK1 在 Peptidoglycan 刺激巨噬細胞 RAW264.7 引發環氧酵素-2 表現訊息傳遞路徑之角色探討

Studies on the Roles of Ras/PI3K/Akt and p38 MAPK/MSK1 in the Signaling Pathway of Peptidoglycan-Induced Cyclooxygenase-2 Expression in RAW264.7 Macrophages

中文摘要

Peptidoglycan (PGN)革蘭氏陽性菌細胞壁上的主要成份，與大家所熟知的革蘭氏陰性菌細胞壁成分 lipopolysaccharide (LPS)一樣皆可造成發炎反應的物質，在免疫反應方面扮演著重要的角色。我們先前的研究已知 PGN 在 RAW 264.7 巨噬細胞誘導環氧化酶-2(cyclooxygenase-2, COX-2)的表現，可經由 Ras/Raf-1/ERK 及 NF-kB 的訊息傳遞而來(J. Biol. Chem., 279: 20889-20897, 2004)。本論文主要探討主題爲 Ras/PI3K/Akt 及 p38 MAPK/MSK1 在 PGN 誘導 RAW 264.7 巨噬細胞 COX-2 蛋白表現中所扮演著的角色。PGN 誘導 COX-2 表現會被 PI3K 抑制劑(wortmannin, LY 294002)，Ras 抑制劑 manumycin A，Akt 抑制劑 1L-6-Hydroxymethyl-chiro-inositol-2[(R)-2-O-methyl-3-octadecyl-carbonate](Akt inh.)，p38 MAPK 抑制劑(SB 203580)和 MSK1 抑制劑(Ro 318220)所抑制。而且 PGN 會有時間相關性誘導 IKK alpha/beta，p38 MAPK 和 MSK1 的活性及 Akt Ser473、p65 Ser536 和 Ser276 磷酸化的現象。加入 manumycin A,LY 294002 及 Akt inh.皆可抑制 PGN 誘導 Akt Ser473 和 IKK alpha/beta 磷酸化之現象，但是加入了 manumycin A, LY 294002 和 Akt inh.，並不會抑制 PGN 誘導 p65 Ser536 的磷酸化。此外， p38 MAPK 抑制劑 SB 203580 不同的濃度也不會抑制 PGN 誘導 p65 Ser536 的磷酸化；相反的， p38 MAPK 抑制劑 SB203580 和 MSK1 抑制劑 Ro 318220，卻可以抑制 PGN 所誘導 p65 Ser276 磷酸化的現象。我們也利用轉染及報告基因的方法證實 wortmannin,LY 294002, Akt inh, SB 203580 及 Ro 318220 皆可以抑制由 PGN 誘導 kB-luciferase 的活性。接著我們更進一步證實 PGN 可以依循時間相關性的誘導 p85 alpha 及 Ras 與 toll-like receptor 2 (TLR2)的結合。綜合以上的實驗結果，我們可以推測出 PGN 可經由 Ras/PI3K/Akt 的路徑活化 IKK alpha/beta 再活化 NF-kB 來調控 COX-2 蛋白表現，且又可經由 p38 MAPK/MSK1 這一條路徑經由 p65 Ser276 的磷酸化，同樣活化 NF-kB 來調控 COX-2 蛋白表現。

英文摘要

Peptidoglycan (PGN), a cell wall component of Gram-positive bacteria,can induce multiple similar responses in immune system as same as lipopolysaccharide (LPS) which is well known as a main cell wall component of Gram-negative bacteria caused

inflammations. Our previous study shown that PGN induced cyclooxygenase-2 (COX-2) expression via Ras/Raf-1/ERK and NF- B signaling pathway in RAW 264.7 macrophages (J. Biol. Chem., 279:20889-20897, 2004). In this study, we investigated the roles of Ras/PI3K/Akt and p38MAPK/MSK1 on PGN-induced COX-2 expression in RAW 264.7 macrophages. PGN-induced COX-2 expression was inhibited by specific inhibitors of PI3K (wortmannin and LY294002), Ras inhibitor (manumycin A), Akt inh.
(1L-6-Hydroxymethylchiro-inositol-2[(R)-2-O-methyl-3-O-octadecylcarbonate], p38 MAPK inhibitor (SB 203580) and MSK1 inhibitor (Ro 318220). Moreover, PGN induced IKK alpha/beta, p38 MAPK and MSK1 activity and increases in phosphorylation of Akt Ser473, p65 Ser536 and Ser276 in a time-depenednt manner. PGN induced increases in phosphorylation of Akt Ser473 and IKK alpha/beta on PGN-induced was inhibited by manumycin A, LY 294002 and Akt inh.. However, manumycin A, LY 294002 and Akt inh. did not inhibit PGN-induced p65 Ser536 phosphorylation. Inversely,PGN-induced p65 Ser276 phosphorylation was reduced by SB 203580 and Ro 318220 in RAW 264.7 macrophages. Moreover, PGN-induced kB-luciferase activity was inhibited by wortmannin, LY 294002, Akt inh., SB 203580 and Ro 318220. Further studies reveals that PGN induced the recruitment of p85 alpha and Ras to Toll-like receptor 2 (TLR2) in time-dependent manner. This propose demonstrate that two pathway
cross-link between Ras/PI3k/Akt and p38 MAPK/MSK1 to induce
IKK alpha/beta activity, p65 Ser536 and Ser276 phosphorylation and NF-kB activation, respectively and ultimately induce COX-2 expression in RAW 264.7 macrophages.