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• 計畫中文名稱	革蘭氏陽性菌細胞壁成分 Peptidoglycan 刺激巨噬細胞前發炎物質釋放之訊息傳遞探討(I)
• 計畫英文名稱	Studies on the Proinflammatory Mediators Release Induced by Peptidoglycan from Gram-Positive Organisms in RAW264.7 Macrophages (I)
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• 英文關鍵字	Macrophages; NF-kappaB; Signal transduction; Cyclooxygenase-2 (COX-2); Peptidoglycan(PGN);Ras/Raf-1/ERK
• 中文摘要	在本計劃中，我們將探討革？氏陽性菌細胞壁成分 peptidoglycan(PGN)誘導 RAW 264.7 巨噬細胞 cyclooxygenase-2(COX-2)表現之訊息傳遞？徑。PGN 依劑？及時間相關曲線增加 COX-2 的表現，而這個作用可被 Ras 抑制劑(manumycin A)、Raf-1 抑制劑(GW 5074)及 mitogen-activated protein kinase kinase MEK)抑制劑(PD 098059)所抑劑。在 RAW 264.7 巨噬細胞給予 PGN 可以依照時間曲線活化 Ras、Raf-1 及 ERK。PGN 所誘導增加 Ras 的活性可被 manumycin A 所抑制； PGN 所誘導增加 Raf-1 的 Ser-338 磷酸化可被 manumycin A 及 GW 5074 所抑制； PGN 所誘導增加 ERK 的活性可被 manumycin A、GW 5074 及 PD 098059 所抑制。RAW 264.7 巨噬細胞給予 PGN 可以增加 IkappaB kinase alpha/beta(IKKalpha/beta)和 IBalpha 磷酸化及 IBalpha 的？解及 kappaB-luciferase 的活性。巨噬細胞給予 NF-kappaB 抑制劑(pyrrolidine dithiocarbamate, PDTC)、IkappaBalpha 磷酸化抑制劑(Bay 117082)及 IkappaB protease 抑制劑(L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) and calpain inhibitorI)皆可抑制 PGN 誘導 COX-2 的表現。PGN 誘導 IKKalpha/beta 和 kappaB-luciferase 的活性也會被 Ras dominant negative mutant (RasN17)、manumycin A、GW5074 和 PD 098059 所抑制。？進一步探討發現在 PGN？激可以依照時間作用使 p85alpha 及 Ras 結合至 Toll-like receptor 2 。因此在本計劃中首先發現 PGN 可以經由 Ras/Raf-1/ERK 的？徑？活化 IKKalpha/beta 及 NF-kappaB，進一步誘導巨噬細胞 COX-2 的表現。
• 英文摘要	In this study, we investigated the signaling pathway involved in cyclooxygenase-2 (COX-2) expression caused by peptidoglycan

(PGN), a cell wall component of the gram-positive bacterium, Staphylococcus aureus, in RAW 264.7 macrophages. PGN caused dose- and time-dependent increases in COX-2 expression, which was attenuated by a Ras inhibitor (manumycin A), a Raf-1 inhibitor (GW 5074), and a mitogen-activated protein kinase kinase (MEK) inhibitor (PD 098059). Treatment of RAW 264.7 macrophages with PGN caused time-dependent activations of Ras, Raf-1, and extracellular signal-regulated kinase (ERK). The PGN-induced increase in Ras activity was inhibited by manumycin A. Raf-1 phosphorylation at Ser338 by PGN was inhibited by manumycin A and GW 5074. The PGN-induced increase in ERK activity was inhibited by manumycin A, GW 5074, and PD 098059. Stimulation of cells with PGN activated IkappaB kinase alpha/beta (IKKalpha/beta), IkappaBalpha phosphorylation, IkappaBalpha degradation, and kappaB-luciferase activity. Treatment of macrophages with a NF-kappaB inhibitor (pyrrolidine dithiocarbamate, PDTC), an IkappaBalpha phosphorylation inhibitor (Bay 117082), and IkappaB protease inhibitors (L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) and calpain inhibitor I) all inhibited PGN-induced COX-2 expression. The PGN-mediated increase in the activities of IKKalpha/beta and kappaB-luciferase were also inhibited by the Ras dominant negative mutant (RasN17), manumycin A, GW 5074, and PD 098059. Further studies revealed that PGN induced the recruitment of p85alpha and Ras to Toll-like receptor 2 (TLR2) in a time-dependent manner. Our data demonstrate for the first time that PGN activates the Ras/Raf-1/ERK pathway, which in turn initiates IKKalpha/beta and NF-kappaB activation, and ultimately induces COX-2 expression in RAW 264.7 macrophages.