

• 計畫中文名稱	Toll-Like Receptor 2-Mediated Inflammatory Mechanisms Induced by Peptidoglycan-研究複合體 p85alpha/c-Src/p47phox 的形成在 PGN 誘導 NADPH Oxidase 活化過程中所扮演的角色		
• 計畫英文名稱	Functional Association of P85alpha, C-Src, and P47phox in PGN-Induced Nadph Oxidase Activation		
• 系統編號	PC9706-0855	• 研究性質	基礎研究
• 計畫編號	NSC96-2320-B038-029-MY3	• 研究方式	學術補助
• 主管機關	行政院國家科學委員會	• 研究期間	9708 ~ 9807
• 執行機構	臺北醫學大學醫學研究所		
• 年度	97 年	• 研究經費	1620 千元
• 研究領域	基礎醫學類		
• 研究人員	林建煌		
• 中文關鍵字	?聚醣(Peptidoglycan ; PGN) ; 類鐸受體 2 (Toll-like receptor 2 ; TLR2) ; MyD88 ; NADPH 氧化? (NADPH oxidase) ; 巨噬細胞(macrophages).		
• 英文關鍵字	Peptidoglycan (PGN) ; Toll-like receptor 2 (TLR2) ; Myeloid differentiation protein 88 (MyD88) ; NADPH oxidase ; macrophages.		
• 中文摘要	<p>肽聚醣 (peptidoglycan, PGN) 是革蘭氏陽性菌細胞壁的主要成分，它會活化宿主的免疫系統，並誘導發炎物質的釋放。PGN 會透過與 Toll-like receptor 2 (TLR2) 結合併傳遞 NF- B，然而 PGN 如何誘導細胞內的訊號傳遞徑還有許多待釐清的地方。目前已知 MyD88 是 Toll-like receptor 引發 NF- B 活化的重要路徑。許多報告也指出，MyD88 缺陷的小鼠完全無法刺激發炎細胞激素的產生。我們之前的研究已證實在 RAW264.7 巨噬細胞，PGN 會誘導 TLR2 與 p85 的結合，進而活化 Ras/Raf-1/ERK/IKK / /NF- B 訊號傳遞 (Chen et al., 2004, J. Biol. Chem. 279: 20889)。共同免疫沉澱法 (Co-immunoprecipitation assay) 的實驗我們發現 PGN 會誘導 TLR2, MyD88 和 p85 彼此的結合。最近的研究指出，以細菌之 flagellin 刺激人類結腸上皮細胞，TLR5 會透過 MyD88 與 p85 結合。因此 MyD88 可能是 TLR2 與 p85 結合的橋樑。過去的文獻也指出，利用 pull-down 實驗證實 p85 可與 c-Src 直接交互作用。我們之前的研究也發現 PGN 會誘導 c-Src 的磷酸化、NADPH 氧化酶的活化、和 ROS 的釋放。而 PGN 所誘導的 ROS 釋放可以被顯性抑制突變型的 c-Src 及 NADPH 氧化酶抑制劑所抑制。共同免疫沉澱法的結果 phox 也發現 PGN 會刺激 TLR2, c-Src 和 NADPH oxidase 的次單元 p47 合。因此我們假設 PGN 誘導 NADPH oxidase 的活化，可能是透過 TLR2/ phox MyD88/p85 c-Src/p47 的訊號傳遞 PGN phox 交所誘導的 NADPH oxidase 活化是經由 TLR2 透過 MyD88 與 p85 , c-Src 和 p47 互作用形成一個有功能的蛋白複合體。如果這個假說是正確的，減少 phox TLR2/MyD88/p85 /c-Src/p47 蛋白複合體的形成，應在抑制革蘭氏陽性菌感染的治療上具有重大的價值。在這個子計畫中，我們將去檢視以下的三個假說: phox 假說 1: PGN 誘導 NADPH oxidase 活化途徑中，p85 /c-Src/p47 彼此的結合是透過 MyD88 的路徑。 phox 假說 2: PGN 誘導蛋白複合體 p85 /c-Src/p47 的形成是需要透過蛋白</p>		

domain-domain 之間彼此的交互作用。 phox 假說 3: 利用表現 p85 , c-Src, 或 p47 的剔除或突變蛋白或者利用針對它們的 siRNA 將會破壞 PGN 所誘導的訊號傳遞 此計劃的整體目標是去釐清革蘭氏陽性菌細胞壁成分 PGN 誘導發炎反應的分子機制，以期發展有效的策略來治療革蘭氏陽性菌的感染。

• 英文摘要

Peptidoglycan (PGN), a Gram-positive bacterial cell wall component, activates the host immune system and induces release of inflammatory mediators. PGN binds the Toll-like receptor 2 (TLR2) and conveys signals to activate NF- κ B. However, the intracellular signaling events following TLR2 activation by PGN are largely unknown. To date, it is known that NF- κ B activation by TLRs is primarily mediated via MyD88-dependent pathway. Several reports indicated that macrophages from MyD88-deficient mice are completely defective in the production of inflammatory cytokines. A recent study from our laboratory shows that PGN induces the association of TLR2 with p85 α resulting in the activation of the Ras/Raf-1/ERK/IKK α / β /NF- κ B signal pathway in RAW 264.7 macrophages (Chen et al., 2004, J. Biol. Chem. 279: 20889). We also found that PGN stimulation triggered the association among TLR2, MyD88, and p85 α by co-immunoprecipitation (co-IP) assay. Recent studies indicated that TLR5 of human colonic epithelial cells in response to flagellin recruits p85 α by MyD88. Therefore, MyD88 maybe act as a bridge between TLR2 and p85 α . Previous studies have shown that p85 α directly interacts with c-Src by pull-down assay. In a preliminary study, we found that PGN induced c-Src phosphorylation, NADPH oxidase activation, and reactive oxygen species (ROS) release in macrophages. PGN-induced ROS release was attenuated by a dominant negative mutant of c-Src and a NADPH oxidase inhibitor. We also noted that PGN phox by co-IP assay. stimulation triggered the physical association among TLR2, c-Src, and p47 Therefore, we suggest that PGN-induced NADPH oxidase activation may be mediated through phox pathway. The Central Hypothesis of this project is that TLR2/MyD88/p85 α /c-Src/p47 PGN-induced NADPH oxidase activation is mediated by the formation of a functional phox complex of TLR2/MyD88/p85 α /c-Src/p47 in RAW 264.7 macrophages. If this hypothesis is correct, measures directed at decreasing the complex formation among phox TLR2/MyD88/p85 α /c-Src/ p47 may have therapeutic value in the prevention of Gram-positive bacterial infection. In this project, we will test the following 3 hypotheses: phox 1. Hypothesis: MyD88-dependent association of p85 /c-Src/p47 in PGN-induced NADPH oxidase activation. phox 2. Hypothesis: PGN induces formation of the p85 , c-Src, and p47 complex via domain-domain interaction. phox 3. Hypothesis: Deletion mutants and siRNAs of p85 , c-Src, or p47 abolish PGN-induced signaling events. The overall objective of this project is to elucidate the molecular mechanism of PGN-induced inflammation so that effective interventions can be developed to prevent Gram-positive infection.