• 系統編號	RN9309-1339
• 計畫中文名稱	Thrombin 誘導肺部上皮細胞 NF-kB 活化及 IL-8 基因表現分子機轉之研究
• 計畫英文名稱	Molecular Mechanism of Thrombin-Induced NF-kB Activation and IL-8 Expression in Lung Epithelial Cells
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC91-2320-B038-048
• 執行機構	臺北醫學大學呼吸治療學系
• 本期期間	9110 ~ 9207
• 報告頁數	12 頁
• 使用語言	中文
• 研究人員	陳炳常; 林建煌 Chen, Bing-Chang; Lin, Chien- Huang
• 中文關鍵字	凝血酵素; 蛋白酵素活化受體; 發炎; 間白素; 核轉錄因子
• 英文關鍵字	Thrombin; Protease-activated receptor (PAR); Inflammation; Interleukin-8; NF
• 中文摘要	Thrombin 爲 serine protease 成員之一，它可以促使血小板凝集及誘導肺部發炎反應。本計劃將探討在 A549 肺部上皮細胞中， thrombin 誘導 NF-κB 的活化及 interleukin-8 (IL-8)表現之機轉。Thrombin 可依照時間依賴增加 IL-8 釋放及 IL-8-luciferase 的活性。Thrombin 誘導 IL-8 的釋放可被 NF-κB 抑制胜、Bay117082(IκB 磷酸化抑制劑)、TPCK(IκB protease 抑制劑)所抑制。A549 細胞給予 thrombin 可依時間依賴活化 NF-κB-luciferase 的活性。相同地，TRAP1 及 TRAP2 也可以活化 NF-κB-luciferase 的活性，但是 TRAP3 及 TRAP4 卻不行。當細胞給予 thrombin 時，可誘發 IκB kinase α/β (IKKα /β)活性、Iκ Bα 磷酸化、IκBα 降解及 p65 的磷酸化。當細胞給予 PPACK (PAR 受體抑制劑)、U73122(PI-PLC 抑制劑)、BAPTA/AM (細胞內鈣離子螯合劑)、Go 6976 (典型 PKC α, β, γ 抑制劑)及 Bim(PKC 抑制劑)皆可抑制 thrombin 誘導 IL-8 的釋放。再者，PPACK, U73122, Bim 及 BAPTA/AM 也可抑制 thrombin 誘導 NF-κB 的活化。當細胞給予 thrombin 可導致 PKC α 從細胞質轉位至細胞膜而活化。細胞給予特異性 PKCα 抑制劑 Ro32-0432 呈現劑量相關曲線抑制 thrombin 誘導 IL-8 的釋放及 NF-κB 的活化。而 thrombin 增加 Iκ Bα 磷酸化及降解也可被 Ro 32-0432 所抑制。經由以上的結果顯示，在 A549 細胞中，thrombin 可能經由活化 PAR1 的訊息傳遞路徑活化 PKC α 使 NF-κB 活化，最後促使 IL-8 的表現。
• 英文摘要	NF-？HeB, PKC, lung epithelial cell, signal transduction Thrombin, a serine protease, activates platelet aggregation, and induces lung inflammation. This study investigated the signaling pathway involved in NF- ？HeB activation and IL-8 expression caused by thrombin in A549 lung epithelial cells. Thrombin caused time-dependent increases in the IL-8 release and IL-8-luciferase activity. IL-8 release caused by thrombin was separately attenuated by a NF- kB inhibitotr pepetide, Bay 117082 (I kB phosphorylation inhibitor), TPCK (I？HeB protease inhibitors). Treatment of A549 cells with thrombin caused time-dependent activation of NF-kB luciferase. Similarly, TRAP1, and TRAP2 but not TRAP3 or TRAP4 induced NF-kB activation. Stimulation of cells with thrombin caused an increase in the activity of I ？H kB kinase kB (IKK kB]), I kB phosphorylation, I kB kB degradation, and p65 phosphorylation. Treatment of A549 cells with PPACK (a PAR inhibitor), U73122 (a PI-PLC inhibitor), BAPTA/AM (an intracellar calcium chelator), Go 6976 (a classic PKC kB, kB inhibitor) and Bim (a PKC inhibitor) all inhibited thrombin-induced IL-8 release. Furthermore,

PPACK, U73122, Bim, and BAPTA/AM also inhibited thrombin-induced increase in NF-kB activity. Stimulation of cells with thrombin caused an increase in the activity of PKC kB translocation from the cytosol to the membrane. Treatment of A549 cells with a specific PKC kB inhibitor inhibited thrombin-induced IL-8 release and NF- kB activation in a dose-dependent manner. The thrombin-mediated increases in the I kB phosphorylation and I kB degration were also inhibited by the Ro-32-0432 . These results indicate that thrombin may activate the PAR1 signaling pathway to activates PKC kB, which in turn initiates NF- kBactivation, and ultimately induces IL-8 expression in A549 cells.