行政院國家科學委員會專題研究計畫 期中進度報告

酯質筏(lipid raft)相關之訊息傳導在 TRAIL 引起 T 細胞活化共刺激訊息之研究(2/3)
期中進度報告(精簡版)

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執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
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計 畫 主 持 人：許秉寧

報 告 附 件 ：出席國際會議研究心得報告及發表論文

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中 華 民 國 96 年 05 月 30 日

行政院國家科學委員會專題研究計劃成果報告

中文：酯質筏(lipid raft)相關之訊息傳導在 TRAIL 引起 T 細胞活化共刺激訊息之研究

英文：Lipid raft associated signal transduction in TRAIL-induced costimulation of T cells

計劃類別： ■個別計劃 □整合型計劃

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□兩年後可對外提供參考

(必要時，本會得展延發表時限)

執行單位：台大醫學院

中華民國 96 年 5 月 28 日

Abstract

Key words: Lipid raft, TRAIL, costimulation, signal transduction

Previous studies in our laboratory, we have demonstrated that cross-linking of TRAIL by plate-bound recombinant TRAIL receptor, DR4-Fc fusion protein enhanced T cell proliferation and increased IFN-γ production in conjunction with immobilized sub-optimal anti-CD3 stimulatio, indicating the occurrence of co-stimulation effects on T cells via signals transduced through TRAIL (J. Immunol. 167: 1347, 2001) (Arthritis & Rheumatism 50:629, 2004). Thus, in addition to its role in inducing apoptosis by binding to the death receptors, TRAIL itself can enhance T cell proliferation after TCR engagement and signal the augmentation of IFN-γ secretion via a p38-dependent pathway. We further explore the role of TRAIL co-stimulation on T cells activation and the molecular mechanism of signal transduction through TRAIL in T cells. Activation at the TCR of a set of protein tyrosine kinases (PTKs) is an early event in this process. Our preliminary results indicate that protein tyrosine kinase, Lck is directly activated by TRAIL reverse signaling during TRAIL induced costimulation of T cells. We further study the interaction of PTKs and associated molecules in lipid raft during TRAIL induce T cell activation. We also investigated the localization and the association of TRAIL with the PTKs involved in early events of T cell activation, and the expression of raft-associated ganglioside, GM1, in lipid raft domains during TRAIL-induced T cell activation. For further exploration of intracellular signal trasnduction pathways for transducing TRAIL costimulation signals, the downstream major signal trasnduction pathways including PI3/Akt, MAP kinase and NF-κB pathways are investigated to define the signal trasndcution pathways during TRAIL-induced T cell costimulation. We have demonstrated that both PI3K/Akt and NF-κB pathways are involved in the TRAIL-induced costimulation of T cells, and TRAIL engagement could direct activate PI3K/Akt and NF-κB pathways, and this may be via relocalization and assembly of signaling complex in lipid rafts. This study will provide a new approach to address the role and molecular mechanisms of TRAIL induced co-stimulation in T cell activation.

在我們實驗室先前的研究中顯示出當將 TRAIL 的受體，DR4-Fc 重組蛋白 immobilized 在 plate 上，進而把加入的 T 細胞表面上的 TRAIL 分子 cross-link 時；可在低濃度的 immobilized anti-CD3 下造成老鼠脾臟 T 細胞分裂增生，並可使得 T 細胞的細胞素，interferon-γ (IFN-γ)分泌增加。顯示著此種 T 細胞活化的作用是

經由 T 細胞表面上的 TRAIL 分子所引起。因此除了引起引起細胞凋亡之外，TRAIL 也可以在 T 細胞與經由 T 細胞受體的訊息共同造成 T 細胞的活化(J. Immunol. 167: 1347, 2001) (Arthritis & Rheumatism 50:629, 2004)。我們的研究中顯示出 TRAIL 所傳導的逆向訊息可提供 T 細胞活化過程中所需的共刺激訊息(訊息 2)，因而造成 T 細胞的活化。因此我們進一步將探討 TRAIL 在 T 細胞活化上的角色及所傳遞訊息的分子機轉。

T 細胞受體的接觸可在 T 細胞膜上造成一系列複雜的分子反應導致 T 細胞的活化。在這過程中早期經由 T 細胞受體訊息傳導所活化的是 T 細胞膜上一系列的 protein tyrosine kinase (PTK)。在我們初步的研究結果中顯示經由 TRAIL 分子在 T 細胞所引起的活化訊息傳導中可直接活化 Lck。在此計畫中我們將進一步探討 TRAIL 在 T 細胞活化的過程中，PTKs 與相關分子在 lipid rafts 上相互作用的情形。我們將探討 TRAIL 與 T 細胞活化過程中的 PTKs 在 lipid rafts 上的位置與相互作用的情形。以及經由 TRAIL 分子在 T 細胞所引起的活化訊息傳導中 raft-associated ganglioside, GM1 的表現情形以及與 PTKs 及相關分子在 lipid rafts 上相互作用的情形。在進一步探討經由 TRAIL 分子所引起共刺激作用之可能的分子機轉上，我們研究 TRAIL 在 T 細胞可能的傳遞訊息途逕，以及可能與 TRAIL 結合參與訊息傳遞的分子。我們進一步探討 TRAIL 在細胞內引起 T 細胞活化可能的主要傳遞訊息途逕，包括 PI3/Akt, MAP kinase 以及 NF-κB pathways 等。同時我們將採用 siRNA 將 T 細胞活化可能的重要 kinase 加以抑制進而探討這些重要的 kinase 在 TRAIL 所引起 T 細胞活化訊息傳導中所扮演的角色。我們已證實 TRAIL 在細胞內引起 T 細胞活化可經由活化 PI3/Akt 以及 NF-κB pathways 的傳遞訊息途逕，且這可能是經由酯質筏(lipid raft)上的訊息傳導分子重新分布及聚集所導致。我們目前正探討 TRAIL 對於酯質筏(lipid raft)上相關的訊息傳導分子之相互作用與影響。此研究將可對於 T 細胞活化之訊息傳導以及 TRAIL 等分子所引起之共活化作用之分子機轉提供一個新的研究方向。

Introduction

During T cell activation, there are two signals involved in activating T cells. One is the signal transduced through T cell receptor (TCR), the other is the costimulation signal transduced through CD28 or other costimulation molecules. Recently, there are a number of T cell surface molecules with costimulation activity reported including TRAIL (1-9). The significance of these newly defined costimulation molecules to T cell activation is still not clear. TRAIL, a novel member in TNF superfamily, induces apoptosis in transformed cell lines of diverse origin and its expression is upregulated in activated T cells (10-18). Previously, we have shown that triggering of TRAIL by

immobilized TRAIL receptor, DR4-Fc, induced maximal proliferation response and enhanced IFN-γ secretion in mouse T cells (6). Thus, in addition to its role in inducing apoptosis by binding to the death receptors, TRAIL itself can enhance T cell proliferation after TCR engagement. These findings suggest the possibility that TRAIL-induced costimulation may trigger activation of human T cells *in vivo*. Enhanced reactivity of T cells to autoantigens via costimulation might be implicated in the pathogenesis of human autoimmune diseases (19).

For further explore the possible molecular mechanisms of TRAIL-induced T cell co-stimulation, we attempt to study the possible signaling pathway and the TRAIL associated molecules in transduction of TRAIL reverse signal as well as other co-stimulation signals in T cell activation. In order to investigate the initial signal transduction events in plasma membrane, we will study the localization and interaction between TRAIL and Lck as well as other key tyrosine kinases in lipid rafts after engagement with TRAIL receptor on T cell membrane. Lipid rafts are highly ordered cholesterol and ganglioside-rich platforms that can facilitate and coordinate close interactions between critical signaling molecules to amplify downstream signaling (20,21). In normal T cells, ligation of the TCR induces rapid lipid raft clustering that leads to concentration of signaling proteins at the area of contact between APCs and T cells known as the immunological synapse (22, 23). Efficient formation of the immunological synapse is critical to amplification of signals downstream of the TCR and subsequent T cell activation (24). Conversely, loss of integrity of lipid raft processes have been shown to play a major role in the pathogenesis of several diseases such as infections, allergies, neoplasms(25). Alternation of lipid rafts composition and associated signaling have been reported in SLE (26,27), suggesting that lipid rafts associated signal transdcution plays an important role in amplification of signals downstream of the TCR and subsequent T cell activation. In our previous studies, we have demonstrated that TRAIL-induced costimulation is involved in activation of human CD4 T cells and may play a role in enhanced reactivity of T cells to autoantigens in autoimmune disease. Several factors can influence the strength of signals controlled by lipid rafts such as, lipid raft pool size, membrane distribution pattern, protein content, and the kinetics of cytoskeletal rearrangements following T cell stimulation. Thus, we assessed the contribution of lipid rafts to T cell responses during TRAIL-induced costimulation. The changes in lipid raft composition and dynamics may contribute to amplification of TCR-mediated signals during TRAIL-induced costimulation.

For further characterization of the signal transduction pathways for transmitting TRAIL reverse signals, we will assay the major signal transduction pathways during TRAIL-induced costimualtion of T cells. Previous study we have demonstrated that

p38 MAP kinase is activated and to augment the production of IFN-γ during TRAIL-induced costimulation of T cells (10). We will study the PI3/Akt pathway, MAP kinase pathways as well as the NF-kB pathway to define the signal trasndcution pathways during TRAIL-induced T cell costimulation. In addition, we will try to use siRNAs to silence the key kinases in TRAIL-induced costimulation of T cells to define role of these signal transduction pathways in TRAIL costimulation.

This study will provide a new approach to address the role and molecular mechanisms of TRAIL induced co-stimulation in T cell activation. Targeting TRAIL-induced costimulation of T cells represents a new modality to reverse T cell hyper-reactivity and treatment of human autoimmune diseases.

Results and Discussion

Previously, we have shown that triggering of TRAIL by immobilized TRAIL receptor, DR4-Fc, induced maximal proliferation response and enhanced IFN-γ secretion in mouse T cells. Thus, in addition to its role in inducing apoptosis by binding to the death receptors, TRAIL itself can enhance T cell proliferation after TCR engagement. Enhanced reactivity of T cells to autoantigens via aberrant costimulation signals may play a role in the development of human autoimmune diseases. Our results demonstrated that TRAIL induced activation of human CD4 T cells in conjunction with signal from TCR. It raises the possibility that TRAIL-induced costimulation of T cells may occur in human T cell activation, leading to an enhanced reactivity to low affinity self-antigens in autoreactive T cells. We have characterized the T cell subsets responding to TRAIL co-stimulation in T cell activation and to further investigate role of TRAIL induced co-stimulation in the pathogenesis of human auotimmune diseases and we demonstrated that TRAIL costimluate human CD4 T cells and also enhanced the proliferation and IFN-γ production in SLE and RA patients CD4 T cells.

For further explore the possible molecular mechanisms of TRAIL-induced T cell costimulation, we are studying the possible signaling pathway in transduction of TRAIL reverse signal as well as other co-stimulation signals in T cell activation. We investigated the signal transduction pathways in TRAIL-induced T cell costimulation. We used an *in vitro* T cell stimulation system with immobilized anti-CD3 and recombinant TRAIL receptor, DR4-Fc proteins to activate human T cells. We have demonstrated that both PI3K/Akt and NF-κB pathways are involved in the TRAIL-induced costimulation of T cells, and TRAIL engagement could direct activate PI3K/Akt and NF-κB pathways, and this may be via relocalization and assembly of signaling complex in lipid rafts. Our results demonstrated that cross-linking of TRAIL by immobilized DR4-Fc in conjunction with suboptimal anti-CD3 induced phosphorylation of Akt kinase, which could be suppresssed by

Wortmannin, a PI-3 kinase inhibitor and Akt specific inhibitor. The enhanced T cell proliferation and increased IFN-γ production induced by TRAIL costimulation could also be inhibited by transfection with Akt dominant mutant construct or with Akt siRNA to knock down the expression of Akt. Moreover, we also demonstrated that TRAIL engagement directly induced phosphorylation of Akt and its downstream kinase GSK-3 beta in Jurkat cells. These results indicate that PI-3K/Akt signaling pathway is essential in TRAIL-induced costimulation of T cells. This study will provide a new approach to address the role and molecular mechanisms of TRAIL induced co-stimulation in T cell activation. This part of work is writing manuscript for submission for publication.

Moreover, we have also demonstrated that TRAIL-induced costimulation induces NF-κB activation. The TRAIL-induced NF-κB activation is associated with activation of IKK and Bcl-10 pathways, and the activation of PI3K/Akt and NF-κB pathways may be linked with relocaization and assembly of signaling complex in lipid rafts. We are currently working on the activation of lipid rafts associated signaling complex during TRAIL-induced costimulatiopn now. This part of work is under writing manuscript for submission for publication. These results will provide a new vision to address the role of T cell activation and TRAIL induced co-stimulation in the pathogenesis and therapy of human auotimmune diseases.

Self-estimation

We are satisfied with the progress we have obtained in recent one year and we also have fruitful publication in this year. We will keep following the data we obtained, and the results will submit for publication in the near future.

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出席國際會議心得報告
報告撰寫人: 台大免疫所吳怡瑩/許秉寧
會議名稱: 國際細胞素會議
(Cytokines 2006 Molecular Biology & Human Diseases)
會議時間:2006 年 8 月 27 日~2006 年 8 月 31 日
會議地點:奧地利維也納
補助單位：國科會

一.參加會議經過

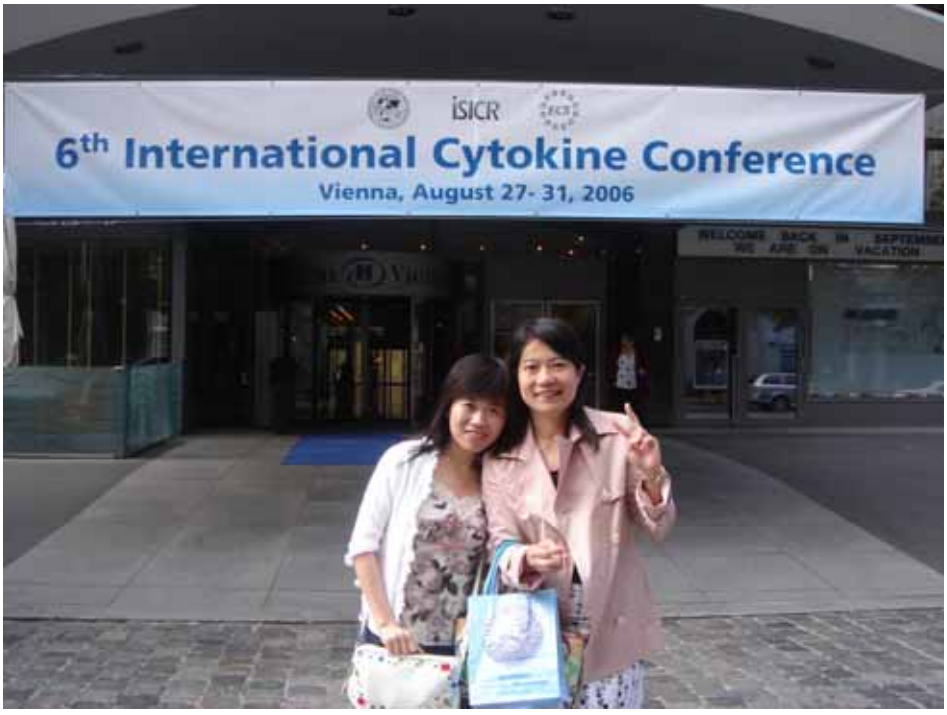
本年度國際細胞素會議是結合國際干擾素與細胞因子學會（International Society of Interferon and Cytokine Research, ISICR), 國際細胞因子學會(International Cytokine Society, ICS)和歐洲細胞因子學會(European Cytokine Society, ECS)三大組織在奧地利維也納的希爾頓飯店舉行。來自38個國家和地區的近400位科學家相聚，其中來自台灣的有８位。

五天會議的安排了包含免疫學、癌症、炎症等不同領域的生物化學、分子生物學、細胞生物學、基因學等，分別以全體大會報告、分會報告、海報展示等不同的形式進行學術的探討和交流。

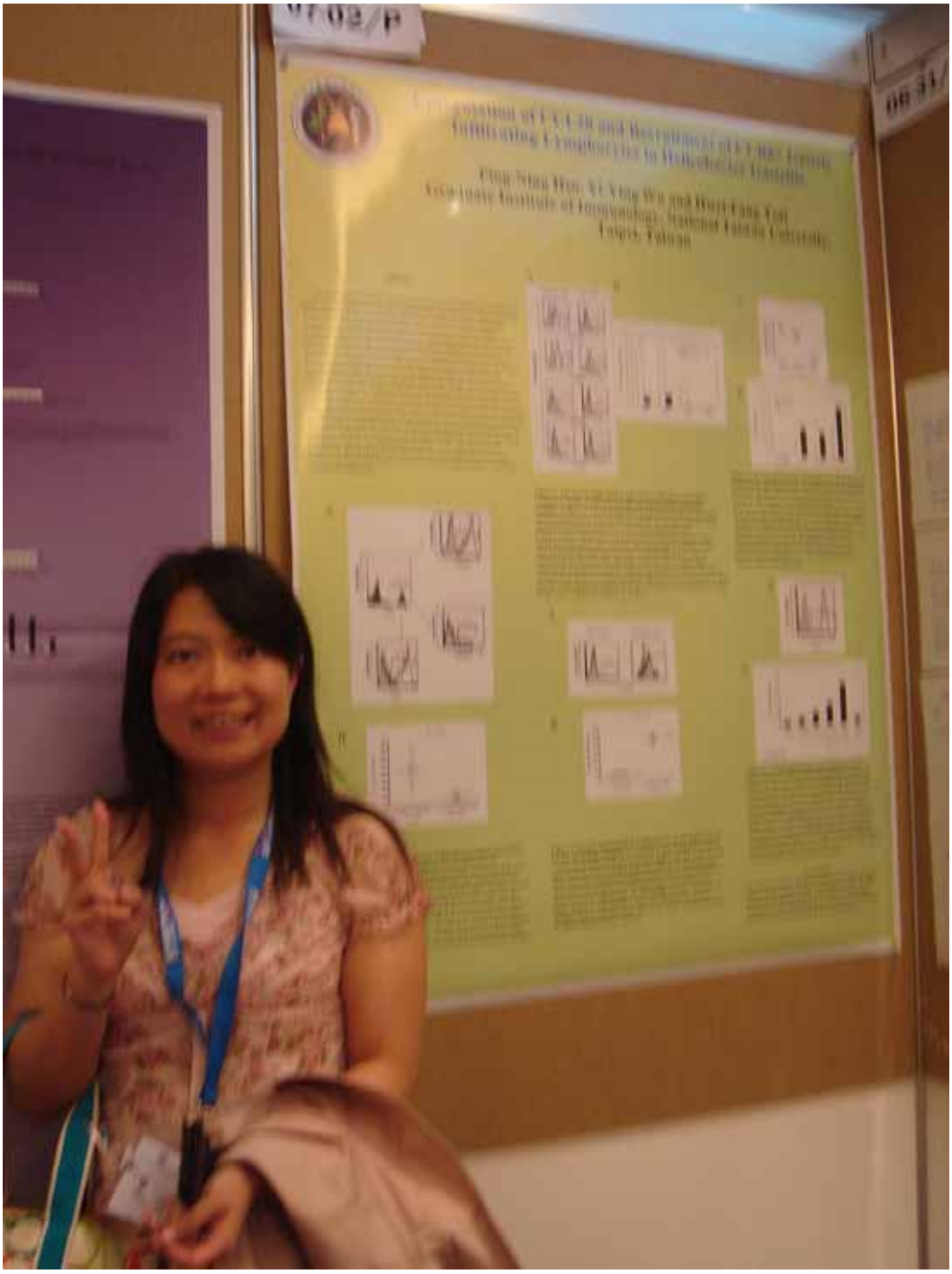
本次會議邀請了許多世界知名的科學家來到會議現場演講，會議開幕式先請到洛克斐勒大學（Rockefeller university）的 Darnell 博士來回顧整個干擾素發現的歷史和研究的過程以及目前研究的方向及未來的展望。干擾素是一種蛋白質，當人體在遇到病毒侵入時，會在人體自動產生。它是人體內對抗病毒入侵的最快反應。干擾素主要分成三大種類：α、β、γ。Darnell 博士畢生致力於研究干擾素所引起的訊息傳遞途徑中重要的訊息傳遞分子，本次會議 Darnell 博士的演講主要著重在干擾素下游分子 STAT1 的去活化調控機制，Darnell 博士指出，STAT1 分子可以藉由結構的轉變，方便去活化的反應。在這次國際會議中，見識到國際會議緊湊的節奏以及各國學者的風範，在會議現場中，大家提問及討論均相當踴躍，而演講者對於自己研究都非常的堅持，提出問題的聽眾所提的問題都直接切中要點，在一來一往的討論中可以見識到科學家對於專業領域的專精，與邏輯推理的能力，我不禁領悟到，科學就是如此的進步的，是藉由不斷的提出問題討論以及和各國的研究工作者互相交流分享。學生亦於會中以海報的方式呈現論文，並簡短的用英文說明自己的研究成果，報告題目是 Upregulation of CCL20 and Recruitment of CCR6+ Gastric Infiltrating Lymphocytes in Helicobacter Gastritis，得到許多教授寶貴的意見，其中一位任教於澳洲雪梨大學的老師非常讚賞，主動留下我的連絡方式，對未來的研究方向有極大的幫助。

二. 與會心得

感謝國科會及學校的支持與贊助，讓學生可以順利參加第六屆國際細胞素會議，並以製作海報的方式以英文口頭報告我的研究成果，這次會議的與會人員多半是歐洲科學研究者，雖然大會溝通官方語言是以英文為主，但其實各式各樣的口音都有，本來很擔心自己的語言能力有所不及，幸而在指導老師許秉寧副教授五年的訓練之下，讓我能從容不迫的報告完我所要講的內容，並且能盡己所能回答各國學者的問題，除了報告自己的研究成果外，我也聽取來自世界各國研究人員的報告，欣賞大師級學者精采的演說，也從和各國學者的互動中，對於自己研究方向有許多啟發，一直知道英語的重要性，但經由這次的國際會議才有深深的體悟，在國際會議場合，重要的就是在短暫的時間內把握機會各研究人員做有效的溝通，所以如果英文語法錯誤百出，或者英文發音不正確，就無法有效的傳達自己的想法給對方，當然也就無法獲得對方給予自己研究方向的建議，而在國際會議若不能與他人討論，其實就沒有到達現場的必要，經由此次會議，我除了思索日後研究方向外，另外也體認到在自己語言能力上應該要更加強，以期日後出席際會議時能和其它國外學者更有效的溝通



圖一　國際細胞素會議會場，維也納希爾頓飯店



圖二　學生於會議中發表研究成果的海報