國軍軍醫人員因公出國參加會議報告書

（出國類別：參加學術會議）

2010 國際免疫學大會(International Conference of Immunology , ICI)

奉准文號：國防部國醫保健字第990000114號

單　　位：國防醫學院預防醫學研究所

報 告 人：上校組長王有欽

出國地點：日本 神戶市

出國時間：自99年8月21日至99年8月28日

報告日期：中華民國 99 年 9 月 28 日

摘　　要

國際免疫學大會(International Conference of Immunology ,以下簡稱　ICI) 是一個世界性的免疫學術研討會，每三年舉辦一次; 今年為第十四屆，在日本神戶市舉行。今年出席這次大會約有來自 76個國家和地區共 6000人，大會為期6天; 包括5天全天候，超過4000篇學術研討會及壁報展示論文; 是國際免疫學大會一個最成功的紀錄。下次的國際免疫學大會將於2013年在羅馬舉行！

個人專長領域並非免疫學，但因從事新興傳染病的研究，特別是各項傳染性致病原疫苗的研發與生產；自然需對免疫學有基本的認知，才能進行相關的研發與產品評估。 很高興能參與這個國際免疫學研討會，個人參與學術壁報展示，題目為「新型流感H1N1疫苗對小鼠之保護性免疫反應研究」(編號2010-A-2803-ICI)；光是和流感病毒相關的研究題目即超過50個。無論在免疫學或流感病毒的研究、疫苗的研發與製造、疫苗品管與確效的評估、免疫佐劑的使用及效果評估、動物試驗的設計及其免疫反應評估、疫苗申請進入臨床試驗程序等均有很大的進展與創意，對於參加者有很大啟發與助益。

（參加**2010**國際免疫學大會會議）

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國防醫學院出國報告審核表

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說明：

一、出國計畫主辦機關即層轉機關時，不需填寫「層轉機關審核意見」。

二、各機關可依需要自行增列審核項目內容，出國報告審核完畢本表請自行保存。

三、審核作業應儘速完成，以不影響出國人員上傳出國報告至「出國報告資訊網」為原則。

壹. 會議緣起

國際免疫學大會(International Conference of Immunology ,以下簡稱 ICI) 是一個世界性的免疫學術研討會，每三年舉辦一次; 今年為第十四屆，在日本神戶市舉行。今年出席這次大會約有來自 76 個國家和地區共 6000 人，大會為期 6 天; 包括 5 天全天候，超過 4000 篇學術研討會及壁報展示論文; 是國際免疫學大會一個最成功的紀錄。下次的國際免疫學大會將於 2013 年在羅馬舉行！

高病原性禽流感病毒H5N1持續發生於鳥類及其在受感染人類近50%致死率，一再說明防止此病毒流行的重要(Subbarao & Joseph, 2007)。 尤其過去幾年有許多禽類直接傳染人類的流感病例報告 (Peiris et al., 2004; Fouchier et al., 2004; Koopmans et al., 2004), 包括幾個亞洲國家發生的高致病性H5N1流感病毒; 至2005年1月26日為止已有至54人感染, 其中41人死亡 (WHO, 2005)。許多專家咸認為禽流感病毒在人類間流行是必然的,只是時間早晚而已。除增進調查及診斷方法外，發展抗病毒藥物及有效疫苗是全世界防疫

努力的目標。

行政院衛生署疾病管制局於 2005 年 4 月從位於英國的 WHO 參考實驗室 (NIBSC) 引自疫苗種 NIBRG-14(A/VietNam/1194/2004)，因為國內民間廠商缺乏流感疫苗自製能力，目前正委託國家衛生研究院疫苗研發中心以雞胚蛋及細胞培養演練生產技術，以備不時之需。 因為流感疫苗自研發至生產均十分重要,自 2006 年起國家衛生研究院於台南舉行年度流行性感冒病毒專門研討會,今年為第三次的年度研討會。 會議主要目的是透過邀請國內外流感病毒相關研究之專家學者演講來提升國內流感病毒之研究及生產的能量。

貳. 參加目的

參加會議主要目的是想透過聆聽國內外免疫學及流感病毒相關研究之專家學者演講來提升自己在免疫學及流感病毒方面之研究及生產的知識,並經由知識產生正確的態度,因而表現出積極之行為;在知識即力量的年代,參加相關研討會可吸收新知,產生正向研發能量,避免閉門造車。

參. 會議過程

時間: 99 年 8 月 22 日下午 5 點~ 99 年 8 月 27 日下午 5 點

地點: 日本 神戶市

議題: 包含 Keynote Lecture, Master Lectures, Symposia, International Symposium on Virus Epidemic; Influenza and Food-and-Mouth Disease, Special Symposium for Clinicans, Lunchtime Lectures, workshops, Poster Secessions 等部份

個人謹就每天較有興趣的研討會進行摘要報告，同時將此次大會中和流感病毒有關的報告摘要彙整，節錄於後:

開幕(99/8/22)

一、主題演講(Keynote Lecture)

MicroRNA's and the Immune System (小分子 RNA 及免疫系統)

David Baltimore (1975 年諾貝爾生理生理學得主)等人

California Institute of Technology, Pasadena, CA, United States

激活哺乳動物的先天或後天免疫反應必須經過嚴格的調控機制，通過精心控制其發生和終止。小分子 RNA 被視為控制不同的生物過程的負調節者，主要作用在轉錄抑制後的水平。人類單核細胞中發現的小分子 RNA 表現圖譜超過 200 個，其中若干（miR-146a/b, miR-132, and miR-155）為內毒素反應的基因。分析 miR - 146a 和 miR - 146b 的基因表達模式，推出了針對各種微生物成分和炎性細胞激素反應的不同型態。透過這啟動子的分析，顯示 miR - 146a 是一個 NF –κB 依賴性基因。重要的是預測 miR - 146a/ b 在與腫瘤壞死因子受體相關因子 6(TNF receptor-associated factor)及 IL - 1 受體相關激酶 1 基因的 3'端非翻譯區鹼基序列配對，研究發現，這些非翻譯區抑制相關性報導基因的表達。這些基因編碼兩個位於 Toll 樣受體和細胞因子下游的關鍵適配器分子。因此，講者提出了一個 miR - 146 在控制 Toll 樣受體和細胞因子信號通過的角色: 一個涉及下調腫瘤壞死因子受體相關因子 6(TNF receptor-associated factor)及 IL - 1 受體相關激酶 1 基因負反饋調節環路徑。

二、每天之大師講座及專題討論會

第 1 天 (99/8/23)

Master Lectures (大師講座)

ML01 M. IL-6: Back to the future (IL6: 過去和未來)

T. Kishimoto (本次研討會會長)

Laboratory of Immune Regulation, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

Chairperson: Marc Feldmann

IL - 6 最初確定為 T 細胞源性細胞因子，它誘導 B 細胞抗體的生產。一系列的後續研究發現，血清 IL - 6 在不同組織和細胞具有多面向活動，其表現失調會導致是幾個慢性炎症和造血惡性腫瘤。80kd IL - 6R 的人性化抗體（Tocilizumab）在 RA，賈和 Castleman 氏疾病的治療效果顯著。此抗體即使是抗腫瘤壞死因子響應炎症性疾病也有效。最近，Th17 被證明和自體免疫性疾病的發病機制有關;而 IL - 6 與 TGF -β 是誘導的 Th17 必不可少的。此研究發現了一種新的轉錄因子誘導 Th- 17 所需的，此轉錄因子為 IL - 6 和 TGF –β 所誘導。這種分子，芳香烴受體（AHR）會與 STAT1 和 Stat5 作用; 並

去除 Th - 17 細胞分化時之負向作用。在 Ahr 去除小鼠及 T 細胞特異性 Ahr 缺陷小鼠一樣完全沒有實驗性關節炎。相比之下，Ahr 會和 STAT-1 作用負向調控巨噬細胞中由 LPS 所誘導之過敏性細胞激訴產生 1。因此，Ahr 去除小鼠變得對 LPS 誘導感染性休克超敏感。講者討論了一種 Ahr 調控 STAT1 所產生調節炎症的新途徑。

專題討論會

Innate Immunity 1 (Innate receptors) (SY1-1)

Chairpersons: Shizuo Akira, Ruslan Medzhitov

SY1-1-1 Fine-tuning TLR and NLR signaling (TLR-like receptor 及 NOD-like receptor 訊息傳遞細調)

L. O'Neill

School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

Toll 樣受體（TLR）的首次詳細定義在 90 年代末，從那時起，出現了顯著的進展，我們了解他們在微生物產品感應上的作用。這導致了文藝復興時期免疫學家對先天免疫機制中的興趣。額外的受體家族被發現，特別是在 NOD 樣受體（NLRs）和 RIG - I 能樣受體（RLRs）。 NLR Nalp3 因為對不同的製病原，包括微生物和內源性（例如尿酸晶體和 β-澱粉狀蛋白纖維）反應而得到特別關注，它作為一個 caspase - 1 激活的調控者。 TLRs 在這方面充當誘導前白細胞介素-1β 和 Nalp3 本身，使以後的 caspase - 1 激活和前白細胞介素-1β 處理。講者討論對這些受體系統之新的調控者。他們發現，microRNA - 21 是一種在 TLR4 訊息傳遞中重要的 PDCD4 蛋白調控者，可借由阻斷 NF –κB 而增加 IL10 產生。他們還發現了一種新的後 caspase - 1 - 蛋白質相互作用蛋白，稱為 Rab39a，是白細胞介素-1β 分泌所必需的。最後，他們發現，IAPP(胰島澱粉樣多肽) ，一種在 II 型糖尿病中重要的分子，可激活 Nalp3，且可能是這種疾病中 IL -1β 重要的誘導者。

SY1-1-2 Roles of TLR inducible genes revealed by gene targeting (基因標靶所竭示之可受 TLR 誘導基因的角色)

S. Akira

Department of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan

哺乳動物 Toll 樣受體（TLR）借由認識不同的微生物成分，對先天免疫反應以及隨後引發的適應性免疫反應發揮至關重要的作用。膜結合之 TLRs 可識別微生物細胞表面上和/或內體的成份。TLR 刺激會誘導的多種負責炎症和免疫反應基因的活化。幾種可誘導基因產物可能參與的 TLR 信號傳遞和反應。

既然 TLR 配體已經確定且 TLR 信號通路已知，講者將研究重點放在 TLR 反應的作用者上。他們產生對 TLR 刺激起反應但其功能不明之基因去除小鼠。其中，他們最近發現了一種基因名為 zc3h12a，是一個核酸美酵素，經分析顯示其參與破壞穩定的 IL - 6 和 IL - 12mRNA。 自發研製的基因敲除小鼠產生自體免疫性疾病伴隨著脾腫大和淋巴結腫大。另一個基因是 JMJD3，這是 H3K27 去甲基酵素。研究顯示，該分子參與巨噬細胞 M2 極化作用。

講者討論這兩個不同的表型基因敲除小鼠，以及這些分子的

作用。

SY1-1-3 Crystal structures of the TLR-ligand complexes (TLR 配體複合物的晶體結構**)**

J. Lee

KAIST, Daejeon, Republic of Korea

Toll 樣受體（TLR）借由認得在不同的微生物分子的保守結構模式而扮演先天免疫反應的重要角色。最近的研究顯示晶體模式識別和 TLR 家族的蛋白質激活機制有關。的 TLR1，TLR2 和 TLR4 蛋白的疏水性配體與內部蛋白口袋互動。相比之下，雙鏈 RNA，一個親水配體，與 TLR3 溶劑暴露表面相互作用。配體，脂肽或雙鏈 RNA 的競賽性結合，誘導 TLRs ectodomains 的二聚化成，其所形成二聚體呈現驚人地相似形狀。在這些 "m"形物，在 TLRs 胞外域的 C 端; 涵蓋中間區域。這一觀察說明，即胞外域的二聚化促使胞內 TLR 區域的二聚化而召回胞內信號適配器蛋白質，進而啟動細胞的訊息傳遞。

SY1-1-4 Innate control of adaptive immunity (後天性免疫的先天性調控)

R. Medzhitov

Department of Immunobiology, Yale University School of Medicine, New Haven, CT, United States

在過去的 10 年，儘管已經取得了很大進展，負責活化適應性免疫反應的機制仍不十分清礎。這是現在人們公認，在大多數情況下，誘導適應性免疫反應是依賴於微生物認識模式識別受體; 啟動樹突狀細胞的運作，開始他們的 T 細胞的活化方案。此外，非認識模式識別受體的適應性免疫調控策略可能存在，例如對某些過敏原的作用方式。最後，其他先天免疫識別機制也可能存在，並發揮調控適應性免疫的作用。在眾多的先天免疫識別戰略及其連接適應性免疫反應活化是重點本次演講介紹。

SY1-1-5 The inflammasomes (過敏體)

J. Tschopp

Department of Biochemistry, University of Lausanne, Epalignes, Switzerland

Inflammasomes 是細胞感染後引發活化或壓力導致炎性細胞因子，如白細胞介素-1β，的成熟並參與先天免疫防禦系統的分子平台。inflammasome 活動的失調和人類遺傳和後天炎症性疾病之間的重要相關性，強調免疫反應路徑研究的重要。白細胞介素 1，活性氧（ROS）及 TXNIP 均與 II 型糖尿病（T2D）現象有關。一直以來，認為長期高血糖觸發 IL - 1 的分泌和 IL - 1 -依賴型胰島功能障礙有關，但其中的機理尚不清楚。研究顯示 NALP3 inflammasome 在 T2D 扮演一個重要的角色。先前研究證實在另一種代謝失調疾病-痛風中，NALP3 inflammasome 可推動 IL - 1 的產生。講者認為 NALP3 inflammasome 是一種代謝壓力感應器。

SY1-1-6 Nucleic acid sensing and activation of immune responses (核酸感應和免疫反應激活**)**

T. Taniguchi

Department of Immunology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

由核酸介導跨膜 Toll 樣受體（TLR）和細胞內受體而活化先天免疫反應，是保護和病理學免疫的重要基礎。在哺乳動物中，跨膜模式識別受體 TLR3，TLR7 和 TLR9 分別認識雙鏈 RNA，單鏈 RNA 和 DNA 的高甲基化，而 RIG - I 樣受體（RLRs），稱為 RIG-1 和 MDA5 為細胞內 RNA 的感應受體。 此外，細胞內的 DNA 感應受體，其中包括 DAI，RIG-I/MDA5 和 AIM2 還會引發先天免疫反應。除 AIM2 外，這些受體激活先天免疫反應的標誌是誘導 I 型干擾素，促炎細胞因子和趨化因子。在這種情況下，IRF3，IRF5，IRF7 因為會激活這些基因而受到特別關注他們的關鍵作用。 最近的研究發現，在核酸層次介導的先天免疫反應的激活，其核酸檢測受體的選擇性激活是由高遷移性族蛋白（HMGBs）的感應所致。 總結細胞質基因誘導的先天免疫反應信號轉遞通路和病原體識別受體之間的信號交叉會談有關。

第 2 天 (99/8/24)

Master Lectures (大師講座)

ML03 M.

Cooper. Dept. of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, United States

Chairperson: Toshio Miyawaki

在脊椎動物不同的適應性免疫系統中有許多相似之處，但不同的是 jawless 兌脊椎動物所使用的亮氨酸的重複序列（LRR 類）為基礎的可變淋巴細胞（的 VLR）受體和顎脊椎動物使用免疫球蛋白為基礎的 TCR 和 BCR 受體。 Jawless 脊椎動物有兩種類型的 VLR，VLRA 和 VLRB，多樣化的類型由不同的淋巴細胞群表達。不完整的種系 vlra 和 vlrb 編碼基因部分的氨基和羧基末端 LRR 類上完整的秸稈地區。 淋巴細胞分化過程中數百個鄰近 LRR 側翼序列備隨機選擇做為模板以有步驟方式複製，明智的方式來完成 vlra 或 vlrb 基因。單等位基因 vlra 裝配與一個被稱為的 APOBEC 同源胞苷脫氨酶 1（CDA1）的表現有關，和單等位基因 vlrb 組裝與 CDA2 表現有關。成熟的 VLRA +淋巴細胞對抗原刺激產生回應，但不分泌 VLRB 蛋白質。抗原結合的 VLRB +淋巴細胞增殖

和分化為漿細胞分泌多聚 VLRB 抗體。 VLRA +細胞不能和原生細菌抗原結合，但一但激活則會上調其白細胞介素- 17 同源基因的表達，期受體在 VLRB +細胞優先表達。相反，激活的 VLRB +細胞會上調 IL - 8 的表達而 VLRA +細胞則上調 IL - 8 受體的表達，從而暗示 VLRA +和 VLRB +淋巴細胞是互動的。令人驚奇的相似之處發生在 jawless 脊椎動物的適應性免疫系統和顎脊椎動物的 T 及 B 細胞譜系之間; 引起許多問題，其中一些將在此演示文稿討論。

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Chairperson: Kazuo Sugamura

CD4 T cells: fates and functions (CD4 T 細胞的命運及功能)

原始 CD4 T 細胞分化成不同的“效應”的細胞類型，包括 Th1 /Th2/Th17/iTreg/Tfh 細胞。這些細胞促成免疫系統的保護和調節作用。每種類型的細胞有一個獨特的功能。透過分化過程，這些細胞適應獨特的命運；這是一個非常有趣的研究和過程。此一重要過程中出現相當早。在 1991 年研究證實表

現 Th2 細胞體外分化需要 IL - 4，一種 Th2 細胞的產品。現在認識到不同的細胞類型分化往往涉及內源性細胞激素訊息傳遞，透過特定的 STAT 蛋白和一主控調節轉錄因子的誘導。最近，應用全基因組分析各種分化的 CD4 T 細胞及其前體組蛋白甲基化狀態，顯示主控調節基因經常在“準備”狀態的“非表示”宗族提供一個彈性基礎，作為這些分化的細胞認識的一個重要特點。此外，全基因組分析約束力的主 Th2 細胞調節者 GATA3 的結合，提供了重要的見解。GATA3 會和 Th2 細胞中 4000 基因結合;在其他分化的細胞類型相結合基因的數目逐漸減少,依其基因表現呈比例。然而,即使在 Th17 細胞， GATA3 會和獨特的基因結合並誘導不同之功能，包括關鍵的 Th17 基因的抑制，這說明主調控者不僅促進“expressor”宗族的分化，且積極抑制其他宗族的功能..

專題討論會

SY1-2 Innate immunity 2 (innate cells) (先天性免疫 2)

Chairpersons: Shigeo Koyasu, Caetano Reis e Sousa

SY1-2-1 Anti-fungal immunity (抗黴菌免疫)

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先天檢測病原體能力是多細胞的存在所必要的，可透過受體編碼種系的演化而能識別非自我結構，所謂的“模式識別受體”（簡稱 PRR）。其中一個受體 Dectin - 1，為 II 型跨膜糖蛋白，具單一胞外非典型 C 型糖識別域（CRD）; 及一細胞質尾巴; 擁有免疫受體 tyrosinebased（亞依淡區）的類激活 motif。 Dectin - 1 主要在骨髓細胞表達，並可認得乙（1-3）-聯葡聚醣。研究證明，這種受體會引起細胞對至 B -葡聚醣反應，包括吞噬作用，內吞作用和氧化破裂，可引起生產花生四烯酸和許多細胞因子和趨化因子。這些反應是透過受體細胞質 ITAM-like motif 引發，利用新穎獨特的信號傳導途徑包括涉及激酶 Syk 的互動和與 TLRs 的合作信號傳遞。Dectin - 1 是第一個非 Toll 樣模式識別受體參與誘導保護性免疫反應的一個例子，透過這些活動 Dectin – 1 對疫小鼠和人類的抗真菌免疫扮演著根本性作用。在這裡，獎者提出新的數據表明了合作性的模式識別受體在慢性真菌感染中的重要性。

SY1-2-2 Innate regulation of adaptive immunity by dendritic cells (樹狀細胞對後天性免疫的調控)

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直接感應致病原是突狀細胞（DC）激活一個主要的啟動要素，從而導致適應性免疫。講者一直都在研究突狀細胞（DC）激活的多模式識別途徑。其中一個感應 RNA 病毒感染包括認識病毒的基因組或如病毒般地感染的細胞內小體車廂，並利用 Toll 樣受體（TLR）的家庭成員，包括 TLR9，TLR7，或 TLR3。病毒基因組可以另外被在細胞質由 DExD / H-box 解旋酶，如 RIG - 1 認識，這是由帶有 5'三磷酸鹽的 RNA 激活。最後，一個獨特的途徑包括細胞表面和 phagosomal 真菌被 C 型凝集素透過 Syk 蛋白激酶信號而認識。值得注意的是，其中一些途徑是不僅涉及直接感應病原體，而且會認識自己因伴隨感染可能的改變，如誘導細胞死亡。這些研究有助於建立一個全球性的突狀細胞（DC）激活的受體及訊息傳遞通路圖譜，可應用於癌症和傳染性疾病免疫治療上。

SY1-2-3 NK cells in viral immunity (自然殺手細胞在病毒免疫中的角色)

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自然殺手細胞對控制某些病毒感染，特別是皰疹病毒家族的成員重要的。因應小鼠巨細胞病毒感染，NK 細胞進行克隆擴張，控制病毒複製，並產生長期存在的“記憶”NK 細胞。值得注意的是，這些 NK 細胞顯現許多適應性免疫淋巴細胞共同的特點與能力，包括加強記憶的反應，並提供宿主增強的保護。NK 細胞上 MHC I 類抑制性受體會損害本反應，使 NK 細胞在缺乏 MHC I 類反應抑制受體提供宿主最佳感染防護。

SY1-2-4 Molecular mechanisms mediating the lymphoid stress-surveillance response (促進淋巴細胞壓力-評估反應的分子機轉)

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淋巴細胞通常被認為是從一個高度多樣化的、具主要抗原特異性稀有細胞所選擇出適應性免疫產生。不過，也有寡克隆淋巴細胞，包括亞群伽馬射線三角洲 T 細胞增殖和 NKTcells 似乎能夠快速反應，裝入通常遇到的基團，包括“壓力抗原”。這種反應促進局部組織免疫監視，但此演示展示他們的影響也散發全身。特別是，急性壓力抗原的表達在表皮大幅提高 Th2 型反應巧合 epicutaneous 抗原暴露與影響腫瘤炎症，過敏，自身免疫，並輔助制定。人們普遍猜測，寡淋巴壓力評估部在開發過程中積極挑選激動劑，但這爭議仍然在缺乏實驗支持。此演示文稿提供一份詳細的通路分子的定義，其中小鼠表皮 T 細胞艙積極選定胸腺髓質上皮細胞。除了補充我們了解 T 細胞的選擇，這項研究可以找出新的途徑，其中上皮細胞的調節組織居民淋巴細胞在皮膚和內臟，與生物和臨床影響。

References:

Hayday, A “Gamma delta T cells and the lymphoid

stress-surveillance

response". Immunity. 2009 31:184-96.

SY1-2-5 Natural helper cell: a newly identified innate lymphocyte producing Th2 cytokines (自然的輔助性細胞:新發現產生 Th2 細胞激素的先天性淋巴細胞)

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先天免疫反應在早期的感染是打擊各種微生物重要的反應。自然殺手細胞是天生的淋巴細胞,不像 T 和 B 淋巴細胞,它不表達抗原受體,但很快對病毒感染的細胞活動表現出細胞毒性且處理各種 cytokines. 講者找到一種新型的天然淋巴細胞,存在新的淋巴結構中;與腹腔脂肪組織有關。這些 細胞不表達細胞表面抗原(lineage markers); 但表達 c - Kit, Sca-1, IL-7R 及 IL-33R.。類似的淋巴集群

在人類腸系膜; 我們稱這組織為與脂肪相關淋巴集群 "FALC"。 在 c -kit 及 Sca - 1 的 FALCL 細胞不同於的 f 祖淋

巴細胞和淋巴組織誘導細胞。這些細胞對 IL - 2 反應而增殖並生產大量 Th2 型細胞因子如 IL - 5、IL - 6 和 IL - 13。 IL - 5 和 IL - 6 調節 B 細胞抗體的生產和 B1 細胞自我更新。事實上，在 c -kit 及 Sca - 1 的 FALCL 細胞支持 B1 細胞的自我更新和加強 IgA 的生產。白細胞介素- 5 和 IL – 13 促進過敏性炎症及防範蠕蟲感染。一旦蠕蟲感染和對 IL - 33 反應，c -kit 及 Sca - 1 的 FALCL 細胞會產生大量的 IL - 13，從而導致杯狀細胞增生，一個驅逐蠕蟲出境關鍵的步驟。在小鼠體內缺乏 c -kit 及 Sca - 1 的 FALCL 細胞，對杯狀細胞增生沒有影響。因此，FALC c -kit 及 Sca - 1 細胞是 Th2 型淋巴細胞，在早期的蠕蟲感染階段發揮著重要的先天作用，我們建議這些細胞應稱為“自然幫手（NH）細胞”。

SY1-2-6 NK cells in mucosal immunity (自然殺手細胞在黏膜免疫中的角色)

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NK - 22 細胞是人類 NK 細胞亞群坐落在黏膜相關淋巴組織; 專門反應 IL – 23 而分泌 IL - 22。講者們調查 NK - 22 細胞擴增所需的細胞激素。 IL - 7 維護 NK - 22 細胞生存並回應了 IL – 23 而生產 IL - 22，但不足以引起強勁擴張。將白細胞介素-1β 或 IL – 2 加入 IL-7 可顯著增加 NK - 22 細胞的增殖； 且較存在 IL - 1b 及 IL - 2 強。相對於白介素- 7，連續培養在 IL -1β 和 IL – 2 中促使 NK - 22 細胞激素圖譜改變。白細胞介素 - 1B 推動構白細胞介素- 22 的分泌，這不同於回應 IL – 23 所產生之急性白細胞介素- 22 生產，且在某些細胞中誘導 IL – 17 的產生。 IL – 2 會減少 IL - 22 和 IL - 17 分泌，增加 IFN-γ 和白血病抑制因子（LIF）的產生。連續培養在 IL – 23 中也會誘導 IFN-γ 的產生。 這些結果表明，NK - 22 細胞功能的可塑性，這可能允許肅主靈活應對不同的病原體。最後，講者們發現 NK - 22 細胞會釋放 B 細胞生存因子 BAFF，意謂 NK - 22 細胞對促進 B 細胞介導的黏膜免疫存在一個潛在的作用。

第 3 天 (99/8/25):

Master Lectures (大師講座)

ML08

Lymphocyte homeostasis: To kill or be killed (淋巴細胞平衡：殺或被殺)

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特異性免疫反應中 B 和 T 淋巴細胞使用複雜的基因重排機制，多樣性產生的抗原受體能夠認識廣泛的任何病原。先天及特異性免疫系統細胞結合到受體觸發複雜的細胞內信號通路，導致新的基因轉錄和細胞活化效應。然而監管以避免這些反應不會轉到自我組織免疫系統的，以免產生自體免疫疾病。淋巴細胞的激活需要多個信號和胞間的相互作用。建立細胞識別自身抗原機制容忍自我存在。一旦病原體的威脅得到解決，會分別以程序性細胞死亡和/或壞死，減少免疫系統的細胞群。一旦重新建立良好的健康，留在體內記憶細胞便大幅度減少第二次接觸病原體的影響。在過去的二十年

中，講者們的實驗室一直從事賦予細胞存活或死亡的細胞分子信號通路的研究。講者報告 Fas 在 germinal centre B 細胞存活特殊的作用， CARMA1/Bcl-10/Malt1 複合物在各種亞群的淋巴細胞的分化和生存的功能， IL - 7 在維持 T 細胞效應功能的耐人尋味任務，和 nfil3 對 NK 細胞發展的重要性。

ML09

Topoisomerase 1 involvement in AID-induced S region cleavage and CSR(拓撲異構酶 1 參與活化誘導胞嘧啶核苷脫氨酶導致之 S 區裂解和類別開關重組)

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活化誘導胞嘧啶核苷脫氨酶（AID）在激活的 B 細胞表現，負責體細胞突變（SHM）類別開關重組（CSR）的誘導。由於除了沒體細胞突變 IgM 外缺乏其他所有的亞類抗體，胞嘧啶核苷脫氨酶基因突變導致嚴重免疫缺陷的 II 型 IgM 症候群。關於胞嘧啶核苷脫氨酶功能的關鍵問題包括：a）胞嘧啶核苷脫氨酶如何切割 DNA 以觸發重組和突變;和 b）如何區

分體細胞突變 V 區和類別開關重組的切換區?要啟動類別開關重組，胞嘧啶核苷脫氨酶裂解它位於各免疫球蛋白重鏈恆定區基因和豐富的回文序列 5'端的 S 區。拓撲異構酶 1（Top1）藉切口、旋轉、及重新接合(religating)DNA 的一股而控制 DNA 的超螺旋旋轉。因此 Top1 降低或胞嘧啶核苷脫氨酶過度表現導致基因組不穩定。現在知道藉由 RNA 促成的基因表現降低(knockdown)造成 TOP1 蛋白降低能促進胞嘧啶核苷脫氨酶依賴性 S 區及類別開關重組的增強。此外，胞嘧啶核苷脫氨酶表現抑制 Top1 mRNA 的轉譯，降低其蛋白水平。此外，Top1 減少造成 Sμ 地區 DNA 結構的改變，這可能阻止 Top1 重新接合，造成不可逆轉的 I 裂解。 相同的，以其其特異性抑製劑喜樹鹼(camptothecin)阻斷 S 區均裂解和類別開關重組將 Top1 完全去活性。結果顯示 Top1 負責類別開關重組的 S 區裂解。

第 4 天 (99/8/26):

Master Lectures (大師講座)

ML10

Vaccines that target dendritic cells (樹突狀細胞標的疫苗)

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國際社會進展已經幫助我們開始概念研究證明人類新樹突狀細胞(DC)疫苗的靶蛋白。要充分利用樹突狀細胞的疫苗，我們首先研究其體內的抗原吸收受體，由 DEC 205 開始。相對於非定標蛋白，標定不同的疫苗抗原給攝取受體可以提高展示給 CD4+ T 細胞和 CD8+ T 細胞約 100 倍。抗原捕獲樹突狀細胞啟動免疫力，特定模式識別受體的佐劑也是將樹突狀細胞從正常的角色轉換成免疫耐受所必須。演講者們的第一個臨床試驗產品是一種抗艾滋病毒的人類 DEC 205 抗體，他與合成雙鏈 RNA，聚 ICLC 或 Hiltonol ®融合一起注射。後者具有良好的安全性。在獼猴，佐劑是 anti-DEC-205-gag 融合抗體誘導抗體和 T 細胞的反應必不可少，包括對 CD8+ T 細胞的展示。在小鼠中，poly IC 是優先選擇的佐劑，通過其能力，自非造血細胞促使大且天生的的 I 型干擾素反應。這種在第 I 型干擾素受體上作用，也在先天免疫的樹突狀細胞上作用。在志願者，聚 ICLC 是一個

真正的微生物模仿者，就像一個天生的活病毒疫苗誘導先天的免疫反應。

因此，講者們從中識別和定性抗原攝取，模式識別和細胞激素反應複雜的受體系統。這預示著一個令人興奮的未來，設計所需要的疫苗和了解他們在樹突狀細胞水平的工作。

ML12

Interleukin-1β and the treatment of auto-inflammatory diseases

(白細胞介素-1β 和自體過敏性疾病的治療)

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慢性炎症性疾病可分為兩類：一是“自體免疫”或“自體過敏”。儘管幾乎所有的自體免疫性疾病具有過敏成分，如類風濕關節炎，在自體免疫性疾病，自體過敏性 T -細胞功能失調。阻止之細胞激素是腫瘤壞死因子，干擾素γ和類似 Th1

細胞激素以及 IL-12/23 和 IL - 17。 “自體過敏性疾病”包括一些由於單核細胞功能障礙，每個阻斷白細胞介素-β（IL -1β 的）局部和全身性疾病。 “自體過敏性疾病”，包括 2 型糖尿病，痛風，新的數據證明，骨髓瘤和心臟衰竭也是自體過敏性疾病。最好的例子是家族性地中海熱的表現形式，因為這種疾病包括發燒，漿膜炎和滑膜組織，生化標誌物和血液系統炎症反應的白血球。自體過敏性疾病的共同細胞激素是 IL - 6，因為這細胞激素驅動 CRP 蛋白，血小板和多克隆 B 細胞活化。蛋白質“cryopyrin” 突變產生一些症狀，稱為“cryopyrinopathies”;相同的證候也稱為“Cryopyrin 相關週期症候群”（ Cryopyrin Associated Periodic Syndromes, CAPS），然而，無突變的患者也觀察到相同的生化，血液和臨床疾病。什麼是共同的鏈接？同的鏈接是激活單核細胞加工和分泌 IL -1β 的嚴格控制失調。其中，IL -1β 的加工和分泌是 IL-1β/caspase-1“過敏體” 的一項功能，此過敏體是細胞內的蛋白質，為 IL -1β 分泌導致的產物。自體過敏性疾病細胞激素生物學的例子，揭示了疾病過程中專一性細胞激素的致病作用。

第 5 天 (99/8/27):

Master Lectures (大師講座)

ML14

E. Unanue. Dept. of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, United States

Chairperson: Takehiko Sasazuki

講題: The unsolved mysteries of antigen presentation (抗原提呈未解之謎)

單一的肽類提呈使 II 型主要組織相容性複合（MHC）分子產生了廣泛的多樣性的 CD4 T 細胞。討論聚焦在多樣性是如何產生以及幾個條件如何深刻影響 MHC class II 結合肽類的性質。兩個這樣的條件是構象異構和肽轉譯後的修飾。肽-MHC 聯合體可以生成構象異構體，一些人認為，最不穩定的 - 被稱為 B 型，可在抗原提呈細胞（APC）一些小泡所編輯，其他不會。蛋白質或肽行經抗原提呈細胞（APC）胞內不同途徑後會產生或編輯出不同構象的肽-肌球蛋白重鏈複合物。最終結果是，B 型複合物在蛋白質處理後不會顯示，只有和 APC 與肽有相互作用。因此，自體蛋白質不顯示 B

型構象，從而 T 細胞誘導其逃離負向選擇，其中有幾個例子現在已經確定。在糖尿病 NOD 小鼠有相當數量的自身反應性 CD4 T 細胞為 B 型 T 細胞，可避免胸腺控制和引起自體免疫。這種來自分泌顆粒的肽複合體通常由在胰島朗格漢斯小島的樹突狀細胞提呈。最後，根據 APC 的狀況，肽的轉譯後修飾發生導致新肽的產生，其對 CD4 T 細胞具高度特異性。一個由瓜氨酸變為精氨酸修改，是自身反應 T 細胞發展成獨特的肽-肌球蛋白重鏈複合物的另一個例子。

專題討論會

SY3-5 Inflammation / Cytokines

Chairpersons: Toshio Hirano, Anne O'Garra

SY3-5-1 Transcriptional and epigenetic control of helper T cell Differentiation (輔助性T細胞分化的轉錄和遺傳控制)

J. O'Shea. NIAMS, NIH, Bethesda, MD, United States

輔助性 T 細胞分化是對微生物病原體免疫反應和自體免疫性疾病的發病機制的關鍵。除了 Thelper1（Th1 細胞）和 Th2 細胞，T 細胞的新命運的不斷湧現而 STAT 家族轉錄因子似乎是這些命運的重要調控者。鑑於 STAT4 和 STAT6 是 Th1

和 Th2 細胞的關鍵角色，我們現在知道 Stat5 和 Stat3 分別是 Treg 細胞和 Th17 細胞必不可少的。Stat3 是體現在人類原發性免疫缺陷 hyperimmunoglobulin E 或 Job's 症候群的關鍵作用。大規模的並行定序和芯片基因體免疫沉澱技術改進，迅速提供了 Stat 如何對基因調控新的意見，重要的是他們如何影響輔助性 T 細胞的遺傳修飾。這些研究也提供 Th17s 如何生成新的見解。

SY3-5-2 Regulation of IL-10 production by cells of the innate and adaptive immune response and implications for immune response to pathogens(先天免疫和適應性免疫反應細胞(調節IL - 10的產生及對病原體免疫反應的啟示)

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缺乏IL - 10，免疫反應掃蕩傳染病病原體可通過旺盛，導致了更好的清除病原體，然而，這可以伴隨著免疫病理學，不利於到宿主。 IL - 10是先天和適應性免疫反應細胞表達，說明這個細胞激素可能在不同階段扮演的重要角色;且對抑制

發炎性病原體占有重要的不同解剖位置。報告顯示對慢性感染，T細胞產生IL - 10和IFN - g。 研究發現，生產IL - 10的Th1細胞發展需T 細胞受體（TCR）與致病原的的高結合，持續 MAP激酶ERK的磷酸化和 IL - 12誘導的 STAT4的轉錄因子的激活。重覆高劑量的TCR 觸發和持續 IL - 12的作用，是維持生產IL - 10 Th1細胞所需。雖然 Th1細胞，Th2和Th17細胞分化需要不同STAT的激活，所有T細胞群均需ERK的激活來生產IL - 10。儘管對病原體的保護具有獨特的功能，所有的T細胞需依賴IL - 10的生產來控制過度旺盛的免疫反應，但刑罰是慢性感染。越來越多的研究對IL-10的基因表現有興趣，目前已知若干分子機制參與其中。講者討論最近樹突狀細胞和巨噬細胞及T細胞中調控 IL - 10表達的調查結果，並討論IL – 10在感染中調節免疫反應的功能。

SY3-5-3 Differentiation and functions of ’mononuclear phagocytes’ in vivo (單核吞噬細胞'在體內的分化和功能)

F. Geissmann. King’s College London, London, United Kingdom

單核細胞和巨噬細胞對炎症及與先天免疫反應的調節扮演關鍵作用，而樹突狀細胞則可啟動和調節後天性免疫反應，他們對免疫記憶和耐受性的發展具有核心的功能。單核細胞，巨噬細胞和樹突狀細胞亞群都有一個共同的骨骨髓先祖，MDP（巨噬細胞和 DC 前體），會表現 SCF, M-CSF,及 FLT3-L
受體，及 CX3CR1 趨化因子受體。 最近在小鼠體內實驗方發掘這些細胞群體之間的發展和傳承的關係。 此外，起源，分化的線索，及許多小鼠組織巨噬細胞，單核細胞，樹突狀細胞亞群精確的功能仍待闡明。最近觀察顯示，在巨噬細胞和 DC，某些細胞亞群可以不需依賴骨髓而自主開發和更新。

SY3-5-4 Macrophages and inflammation:mechanisms of survival (巨噬細胞和炎症：生存機制)

A. Celada1,2. 1Institute for Research in Biomedicine, Barcelona, Spain, 2University of Barcelona, Barcelona, Spain

巨噬細胞在炎症發揮關鍵作用。在發病的炎症過程中，這些細胞被激活並具破壞作用。巨噬細胞活化導致消除細菌的能

力增加， 通過釋放細胞激素調控許多其他細胞。然而，過度激活有破壞作用，例如膿毒性休克，可導致多器官功能障礙綜合症候群死亡。在其他情況下，持久性發炎症活動的結果發展出慢性炎症，如類風濕關節炎，牛皮癬和炎症性腸病疾病。為了防止不良影響，已經發展一些機制來控制活化(包括核酸和聚合酶激活)過程中的傷害。

SY3-5-5 Cross regulation of TLR-triggered pro-inflammatory cytokine production (TLR引發促炎性細胞激素產生的交叉調控)

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TLRs透過MyD88或TRIF訊息傳遞通路誘導發炎性細胞激素或第一型干擾素的生產啟動先天免疫反應,。轉錄後修飾的信號分子已被認為是TLR信號通路一個重要的調節方式的。儘管深入的調查，其中TLR信號細密調整的詳細機制仍不清楚。演講中介紹幾個在調控TLR信號發揮重要的作用分子。其中之一是aM integrin CD11b，在單核細胞和巨噬細胞高度

表達。 CD11b對TLR體外和體內反應均都進行負調控。TLR4和ligand結合後通過 PI3K和RapL內到外信號通路激活細胞 CD11b，然後通過激活Src /Syk 而活化 CD11b的反饋，而抑制TLR信號。 Syk和MyD88及TRIF作用，並誘導MyD88及TRIF酪氨酸磷酸化，並經由E3泛素連接酶cbI-b導致MyD88及TRIF的裂解。因此， TLR引發並且活化integrin CD11b的反饋，與MyD88及TRIF信號通路會談，隨後壓抑先天性免疫反應中的TLR的信號

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SY3-5-6 Role of interleukin 6 amplifier in autoimmune diseases and inflammation (細胞介白素6放大器在自體免疫性疾病和炎症的作用)

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細胞激素在免疫反應，自身免疫和炎症中扮演起著關鍵作用。 研究建立敲入(Knock-in)小鼠表達gp130分子(一個IL - 6信號的傳感器)多個變種，結果顯示F759小鼠發展出類風濕性關節炎類疾病（F759關節炎）。 STAT3的活化再F759小鼠增

強; F759小鼠關節炎端賴IL - 6和IL – 17之作用。人類 T細胞白血病病毒1型P40 Tax蛋白增強F759關節炎，說明STAT3和NF - kB的關鍵作用。此外，F759關節炎是依賴於 CD4 + T細胞，雖然在T細胞不需要F759突變。相反的F759突變nonhematopoietic細胞是F759關節炎必不可少的，表明與非免疫組織和免疫系統互動對疾病扮演著關鍵作用。IL - 6是IL - 17A一個關鍵下游的目標。此外，通過激活成纖維細胞轉錄因子NF - kB和STAT3蛋白，細胞介白素- 17A與 IL - 6一起觸發一個IL - 6的正反饋環。由於 IL - 6誘導 Th17細胞，一旦循環開始後，IL - 6基因表達可連續擴增。結果顯示失調的“IL - 6的放大器”起了F759關節炎關鍵作用。由於“IL - 6的放大器”參與實驗性自體免疫性腦脊髓炎，（EAE的）這種情況可能是其他自體免疫性疾病和炎症性疾病一個潛在一般病因的過程。演講者並提出一個解釋組織特異性自體免疫性疾病如何發展的新模式。

三、流感相關研究:

influenza (H5N1)	i36 (WS/PP-003-05)
influenza A virus	ii27 (WS/PP-025b-015), iv33 (PP-069-15)
influenza chemokine,	ii41 (PP-025-102)
influenza vaccination	iv147 (WS/PP-105-05), v29 (WS/PP-105-05)
influenza vaccine	iii48 (PP-049-12), iv37 (PP-069-42)
influenza Virus Infection	i35 (WS/PP-003-01), i63 (PP-007-37)
influenza virus pneumonia	iv120 (PP-085-15)
influenza virus vaccine	iv30 (WS/PP-069-01)
Influenza virus	i36 (PP-003-08), i144 (WS/PP-023-04), i145 (PP-023-11), ii17 (PP-024-57), ii25 (WS/PP-025a-006), ii30 (PP-025-033), iii44 (PP-048-12), iii49 (PP-049-20), iv95 (WS/PP-081-06)
influenza	i35 (WS/PP-003-03), i36 (PP-003-09), i37 (PP-003-10), i143 (WS/PP-023-01), ii26 (WS/PP-025b-012), ii31 (PP-025-041), ii34 (PP-025-056), ii40 (PP-025-094), ii42 (PP-025-105), ii46 (PP-025-127), ii171 (PP-101-13), ii171 (PP-101-15), iii49 (PP-049-19), iii88 (WS/PP-059-03), iii94 (PP-059-40), iv11 (PP-066-22), iv32 (PP-069-13), iv34 (PP-069-24), iv35 (PP-069-29), iv35 (PP-069-30), iv36 (PP-069-34), iv36 (PP-069-36), iv37 (PP-069-37), iv37 (PP-069-40), iv83 (WS/PP-078-01), iv149 (PP-105-19)

禽流感相關研究:

avian flu vaccine	i13 (PP-001-32)
avian influenza virus	iv13 (PP-066-31)
avian influenza	ii29 (PP-025-029)

Superior efficacy of a recombinant flagellin:H5N1 HA globular head vaccine is determined by the placement of the globular head within flagellin (重組鞭毛蛋白優越的療效：決定病毒 HA 球狀頭部疫苗安置在鞭毛球形頭部的效果)

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Transmission of highly pathogenic avian influenza (HPAI) between birds and humans is an ongoing threat that holds potential for the emergence of a pandemic influenza strain. A major barrier to an effective vaccine against avian influenza has been the generally poor immunopotency of many of the HPAI strains coupled with the manufacturing constraints employing conventional methodologies. Fusion of flagellin, a toll-like receptor-5 ligand, to vaccine antigens has been shown to enhance the immune response to the fused antigen in pre-clinical studies. Here, we have evaluated the immunogenicity and efficacy of a panel of flagellin-based hemagglutinin (HA) globular head fusion vaccines in inbred mice. The HA globular head of these vaccines is derived from the A/Vietnam/1203/04 (VN04; H5N1) HA molecule. We find that replacement of domain 3 of flagellin with the VN04 HA globular head creates a highly effective vaccine that elicits protective HAI titers which protect mice against disease and death in a lethal challenge model.

Intranasal delivery of vaccine with beta-glucan formulation contained liposomes (脂質體包含疫苗與 β-葡聚醣的混合物鼻腔給藥之效果)

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Immunostimulatory polysaccharides known as beta-glucans (β-glucans), a Toll-like receptor (TLR) 2 agonist, have been studied for many years. Herein, we investigated whether the addition of a β-glucan formulation to an inactivated avian influenza virus (AIV), which was encapsulated by liposomes and used to intranasally immunize chickens, could enhance the AIV-specific mucosal secretary immunoglobulin A (s-IgA) and/or serum immunoglobulin G (IgG) antibody responses. Naïve chickens were assigned to four groups and intranasally inoculated with the following vaccine formulas: AIV alone, liposomal vaccine, liposomal vaccine mixed with 1 % β-glucan, 0.5 % β-glucan encapsulated liposomal vaccine followed by mixing with a 0.5 % β-glucan (in/out), and normal saline. Three weeks after the secondary immunization, chickens were sacrificed to collect exudates by tracheal/nasal lavage. The adjuvanticity of β-glucan-formulated liposomal vaccines was evaluated by an enzyme-linked immunosorbent assay (ELISA). The β-glucan encapsulated liposomal vaccine followed by mixing with 0.5 % β-glucan (in/out) elicited significant mucosal s-IgA andserum IgG production compared to the liposomal vaccine mixed with 1 % β-glucan. This study demonstrated that β-glucan encapsulated liposomal vaccines can enhance antibody responses in birds upon avian influenza virus infections.

Immunostimulatory RNAi therapeutics against H5N1 avian influenza(H5N1 禽流感的免疫刺激性 RNAi 治療)

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RNA interference (RNAi) is a vital component of the antiviral immune response that is commonly exploited for gene-silencing purposes. Irrespective of gene silencing, certain RNAi molecules contain immunostimulatory motifs that can trigger inflammatory responses via RNA-sensing molecules such as the Toll-like receptors, cytosolic kinases and helicases. Stimulation of these pathways leads to the induction of pro-inflammatory cytokines and type-I interferon, molecules that are integral to the conferring of antiviral resistance.
We are currently developing RNAi-based therapeutics against the highly pathogenic Influenza A virus subtype H5N1, responsible for the global pandemic of 'bird flu'. Our aim is to develop RNAi candidates that (1) silence avian influenza genes; and (2) induce an interferon response that will aid in the protection against H5N1. We have identified several RNAi sequences with immunostimulatory potential, and are currently employing quantitative real time PCR, viral protection assays, haemoagglutination assays, nitrite assays and luciferase-reporter assays to optimize the immunostimulatory potential of anti-influenza RNAi sequences. We are moving towards the in vivo delivery of RNAi-based antivirals and the testing of candidates in chicken embryos.

Key cytokines/chemokines in acute respiratory distress syndrome with avian influenza (H5N1) infection in Vietnamese children (在越南兒童感染禽流感（H5N1）所引發急性呼吸窘迫綜合徵之關鍵細胞激素/趨化因子)

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Patients with highly pathogenic avian influenza A (H5N1) in National Hospital of Pediatrics (NHP, Hanoi, Vietnam) show the fatality rate associated with fulminant ARDS (acute respiratory distress syndrome during their clinical courses [J. Infectious Dis.,2009].The histopathology of these cases demonstrated diffuse alveolar damage in the lung [Jpn. J. of Infectious Dis., 2008.]. However, roles of cytokines/chemokines and activated neutrophils/macrophages is still unclear. Here, we compared concentrations of 10 cytokines/chemokines of pediatrics patients in groups of H5N1-positive and H5N1-negative. And concentration of lysosomal enzyme myeloperoxidase (MPO), which is released from activated neutrophils, was also measured. We compared concentrations of cytokines/chemokines measured by ELISA in plasma and NPA (nasopharyl aspirate) between two groups. In the NPA, the concentration of IL-6sR in H5N1-positive significantly higher than H5N1-negative group, whereas increased IL-8 level in H5N1-negative group slightly decreased in H5N1-positive group. In plasma, levels of IL-12p40 and TNFR2 in H5N1-positive group significantly higher than H5N1-negative, slightly in IL-6, IL-12p70 and IL-6sR. Inversely, increased IL-8 level in H5N1-negative group was not detected in H5N1-positive group. Interferon γ was not detected in NPA and plasma in two groups. In addition, the concentration of MPO significantly higher in plasma in H5N1-positive group, but was not different in NPA between two groups. These results suggest that mainly IL-6sR may contribute in nasal space associated with lung injury. Moreover, IL-12p40 and TNFR2 may be produced from pulmonary space or endothelial cells to circulate in blood, and also MPO may be released into blood from activated neutrophils.

Interleukin-15 is critical in the pathogenesis of influenza A virus-induced acute lung injury (白細胞介素 15 是流感病毒引起的急性肺損傷發病機制的重要因子)

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Object: It is generally accepted that highly pathogenic influenza infection induces excess immune responses that cause lethal lung injury. However, the mechanisms of the lethal pathology after influenza infection are not fully understood. Interleukin (IL)-15 is a pleiotropic cytokine involved in both innate and adaptive immune responses. In this study, we examined role of IL-15 in acute lung injury induced by influenza A virus infection.

Materials and methods: IL-15-knock out (KO) mice or β2-microglobulin (β2m) KO mice with C57BL/6 background and mice depleted of CD8+ T cells by in vivo administration of anti-CD8 mAb were intranasally infected with 500 pfu/mouse of influenza A/ Fort Monmouth/1/47(A/FM/1/47,H1N1, a mouse-adapted strain). Mortality, viral titer and histopathology of the lung were examined. Influenza-specific cytokine production of T cells was analyzed by intracellular cytokine staining method.

Results and discussion: IL-15 KO mice exhibited reduced mortality after infection with influenza virus A/FM/1/47 albeit no difference in the viral titer from control mice. There were significantly fewer antigen-specific CD44+ CD8+ T cells in the lungs of infected IL-15 KO mice and adoptive transfer of the CD8+ T cells deteriorated the survival of IL-15 KO mice following influenza infection. β2m KO mice and mice depleted of CD8+ T cells by in vivo administration of anti-CD8 mAb displayed reduced mortality rate after infection. These results indicate that IL-15-dependent CD8+ T cells are at least partly responsible for the pathogenesis of acute pneumonia caused by influenza A virus.

Development of CTL-inducing peptide vaccine against Influenza A Virus (發展誘導 CTL 對抗甲型流感病毒的肽疫苗)

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Purpose: To develop a broadly protective Influenza vaccine, we focused on cytotoxic T lymphocyte (CTL) because CTL is able to recognize conserved internal viral peptides presented by the class I of infected cells as epitopes. In this study, we designed HLA-A*2402 restricted CTL epitopes derived from H5N1 Influenza A virus internal proteins and evaluated their protective effect against Influenza A virus infection using HLA-A24 transgenic mice (A24Tg).

Methods: Several epitope peptides from H5N1 Influenza A virus internal proteins were selected by CTL epitope prediction programs. To verify immunodominancy of the peptides, in vivo cytotoxicity assay was used. To evaluate protective effect of CTL peptides, A24Tg, in which a human CTL immune system have been reconstituted, were immunized with CTL peptides, then challenged with several Influenza A virus subtypes. After virus infection, the survival rate and the changes in body weight were daily monitored and lung virus titer was measured.

Results and Conclusions: CTL peptide immunized A24Tg mice survived after H5N1 Influenza A virus infection. Body weight loss of immunized mice was not observed after virus challenge. Lung virus titers at infection day 5 of immunized groups were significantly lower than those of unimmunized groups. Furthermore, same combination of CTL peptides were effective for another 2 virus subtypes. So, we have demonstrated that the Influenza A

virus specific CTL without neutralizing antibody induction have protective effect against Influenza A virus infection regardless of virus subtypes. These results provide the basis of CTL-inducing Influenza vaccine development for human use.

Pathogenic mechanism of influenza pneumonia involved in MPO function of neutrophils (流感肺炎致病機制與中性粒細胞 MPO 的功能有關)

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Avian influenza (H5N1) induces fulminant acute respiratory distress syndrome (FARDS) with high lethality. The pandemic influenza (AH1pdm) also shows ARDS pathology under specific conditions. However, precise pathogenic mechanisms of pneumonia triggered by influenza virus have never been fully understood, causing us to limit appropriate therapeutic treatment. To elucidate the influenza-induced pathogenic mechanism, we inoculated mouse-adapted strain (PR8-H1N1) into BALB/c mice, and then plasma, bronchoalveolar lavage fluid (BALF), and lung tissue were obtained to investigate cytokine-chemokine levels in them. In BALF, the sequential increases of Kc (CXCL1), RANTES (CCL5), and MCP-1 (CCL2) were observed from 2 days post infection with large neutrophil recruitment. In this analysis, type I IFNs, known as antiviral cytokines, are also up-regulated in the lung following specific IRF (interferon regulatory factor) inductions. Our data indicated that specific chemokines elicit infiltration of neutrophils followed by inflammation in the lung by influenza infection. We next focused on the role of myeloperoxidase (MPO) from neutrophils in the lung injury using MPO-KO mice. The study showed that influenza-induced pneumonia in MPO-KO mice developed less than that in wild-type mice. In this study, our results suggested that MPO released from infiltrated neutrophils induce lung injury as well as inactivation of influenza inactivation.

Intrinsic defects in B cell response to influenza vaccination in elderly humans (老人接種人類流感疫苗產生之 B 細胞反應的內在缺陷)

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Objectives of this work are to establish biomarkers of aged human B cells and associate these with a biologically relevant immune response, such as to the influenza vaccine. We have previously shown that elderly humans have fewer percentages of CD19+ total B cells, switch memory B cells, and increased percentages of naïve B cells. Activated CD19+ B cells have less E47, AID and Igγ1 circle transcripts (CTs) with age. AID is critical for class switch recombination (CSR) and somatic hypermutation, both necessary for optimal function of immunoglobulin (Ig). More recently we have initiated a series of experiments to measure the antibody response to seasonal influenza vaccination by hemagglutination inhibition assay (HI) and associated this with the isolated/intrinsic B cell response to these antigens (containing adjuvants) *in vitro*. Our results show that the specific AID response of B cells to the vaccine given *in vitro* and the *in vivo* serum HI response to vaccination are both decreased with age.

We are currently pursuing these studies with more subjects and with both seasonal as well as H1N1 vaccine response. These results indicate that these biomarkers (decreased E47, AID, CSR, IgG) could more accurately track optimal immune responses and activity, and could be valid predictors of vaccine and therapeutic agent effectiveness in humans.
This work was supported by NIH AG28586 and AG32576 (BBB)

Nasal influenza vaccines containing pFL and CpG ODN as mucosal adjuvant elicit cross-reactive functional s-IgA antibody (鼻流感疫苗含有體 pFL 和 CpG 寡脫氧核苷酸作為黏膜佐劑引起的 S - IgA 抗體交叉反應功能)
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Our previous study showed that a plasmid expressing flt3 ligand (pFL) and CpG-ODN as a combined double nasal adjuvant induced balanced Th1- and Th2-type cytokines for OVA-specific secretory IgA (S-IgA) antibody (Ab) responses. In this study, we examined what type of cytokine responses are elicited when influenza viruses are employed as antigens (Ags). Further, functional properties of influenza-specific S-IgA Abs induced by nasal double adjuvant were determined after nasal influenza virus-challenge. When BALB/c mice were nasally immunized with A/PR8 (H1N1) and the combined double adjuvant, significant levels of A/PR8-specific S-IgA and IgG Abs responses were induced in saliva and plasma. Intracellular cytokine analyses revealed increased frequencies of both IFN-γ and IL-4 expressing CD4+ T cells in the nasal mucosa of mice given the combined double adjuvant when compared with mice immunized with A/PR8 alone. In order to test the functional properties of influenza-specific Abs, mice were nasally immunized with A/PR8, A/Wuhan (H3N2) or A/Sydney (H3N2) influenza Ag plus pFL and CpG-ODN three times at weekly intervals and were then nasally challenged with A/PR8 virus (40xLD50). Although essentially no cross-protective efficacy was detected in mice that received the nasal A/Sydney vaccine, mice that received the nasal A/Wuhan vaccine showed significant protection against A/PR8. Thus, A/Wuhan vaccinated mice contained significant levels of cross-reactive S-IgA Abs in external secretions. These results suggest that pFL and CpG-ODN as nasal adjuvant induce both Th1- and Th2-type cytokines for the induction of cross-protective S-IgA Ab responses.
Supported by NIH grants AG025873 and DE012242.

Antibody responses to immunization with seasonal and 2009 H1N1 vaccines (季節流感及 2009 年 H1N1 疫苗免疫產生之抗體反應)
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The influenza pandemic virus that appeared in 2009 provided an opportunity not seen in more than 30 years to study responses to an influenza strain that was new to the population in the context of regular seasonal influenza vaccination. Our study involved 10 healthy subjects who were first immunized with the 2009-10 seasonal influenza subunit vaccine, then with the 2009 swine-origin H1N1 subunit vaccine. Four blood samples were obtained: (1) on the day of seasonal vaccine administration, (2) two weeks later, (3) 6 weeks later on the day of H1N1 vaccine administration and (4) two weeks after H1N1 vaccination. Each plasma sample was assayed for three antibody parameters (amount of antibody, overall avidity, and HAI titer) against three viruses; A/Uruguay/716/2007 (the H3N2 component of the vaccine), A/Brisbane/59/2007 (the seasonal H1N1 component of the vaccine) and

A/Oklahoma/3052/2009 (a local 2009 swine-origin H1N1). The results show that some individuals' response to the seasonal vaccine included antibodies that cross-react with the swine-origin H1N1 virus, as shown by increases in amount of antibody, overall avidity, and HAI titer. This cross-reactivity seemed not to be reciprocal, as a significant response to the seasonal H1N1 was not seen after 2009 H1N1 vaccination. Furthermore, in many subjects there was no further increase in titer to the 2009 H1N1 after immunization with the subunit vaccine containing a similar virus. The results suggest that immunization with the seasonal vaccine may have provided some degree of protection against the pandemic virus, at least in our highly immunized cohort.

Conventional DCs provide compensatory mucosal immunity in the absence of plasmacytoid DCs during influenza virus infection (流感病毒感染期間傳統的樹狀細胞會提供缺乏漿樹狀細胞補償性黏膜免疫)

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The complex interplay between dendritic cell (DC) subsets dictates the nature of innate and adaptive mucosal immunity to viral infection. Although plasmacytoid (p)DCs have been implicated in both the control and pathogenesis of influenza virus infection, the relationship of pDCs to conventional (c)DCs during influenza infection has yet to be addressed. To assess the role of pDCs within the mucosal DC compartment, we characterized DC-based antiviral immunity in non-treated controls or mice receiving antibody-mediated pDC ablation during A/PR/8/34 (H1N1) influenza infection. Infection induced rapid expansion of pDCs in the lung and peribronchial lymph nodes (PBLN) with robust concomitant cytokine production. Nevertheless, control and pDC-depleted groups displayed comparable morbidity resulting in sacrifice at six days post-infection (PI). pDC ablation significantly augmented recruitment of cDCs to the lung with an associated increase in the number of bromodeoxyuridine-positive monocyte and pre-DC progenitors, indicating enhanced inflammation-driven cDC differentiation. Intracellular FACS analysis revealed significant increases in TNF-α and IL-6 production in cDCs from pDC-depleted mice over non-treated controls both early and late PI. Notably, proinflammatory cytokine production from cDCs in the absence of pDCs attained levels 50 fold greater than those found in cDCs of controls. Additionally, pDC ablation resulted in early recruitment of $CD11b^{Neg}CD8\alpha^{Pos}$cDCs to the PBLN, suggesting a role for augmented antigenic cross-presentation by cDCs in the absence of pDC support. These findings describe a novel mechanism by which the magnitude and function of the cDC compartment compensates for the absence of pDCs and provide relevant insight into influenza virus pathogenesis.

KLRG1 expression in the lungs by influenza A virus infection in mice (小鼠感染流感病毒肺臟中 KLRG1 之表現)

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It is known that innate immunity in the lung play an important role in the influenza A virus (IAV) infection process. Among immune cells, we have focused on NK cells because NK cells can kill influenza virus-infected cells without prior antigen stimulation. To see how the NK cell functions are regulated, we s investigated KLRG1 expression on NK cells in mouse lungs. Since previous reports indicated that the binding of KLRG1 by E-cadherins, which are

expressed abundantly in the lungs, inhibits NK cell cytotoxicity, the interaction between KLRG1 and E-cadherin likely contributes to lung homeostasis. To clarify the involvement of KLRG1 in the pathogenesis of IAV infection, KLRG1 expression in mice examined. BALB/c mice were infected with influenza A/PR/8 virus by inhalation and cells isolated from the lungs and the spleen were examined immunologically. Virus titer in the lung was also measured. The results showed that the number of KLRG1(+)NK cells was increased in the lungs by A/PR/8 infection, while the expression of KLRG1 on splenocytes was not changed. Further animal studies of blocking KLRG1 function by F(ab')2 fragments of antibody against KLRG1 are under way to reveal the physiological function of KLRG1 during the IAV infection.

Macrophage TRAIL expression is IFN-ß-dependent and mediates alveolar epithelial apoptosis and barrier dysfunction in influenza virus pneumonia (巨噬細胞 腫瘤壞死因子相關凋亡誘導配體(TRAIL)的表達為干擾素 -β-依賴性可導流感病毒性肺炎肺泡上皮細胞凋亡和屏障功能的障礙)

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Influenza virus (IV) pneumonia occasionally progresses to acute lung injury. Although alveolar macrophages (AM) have been attributed a significant role in the host defense towards IV, they were shown to contribute to lung injury progression by release of pro-inflammatory mediators and cytokines such as the pro-apoptotic TNF-related apoptosis-inducing ligand (TRAIL). However, the molecular signals underlying the regulation of macrophage TRAIL and its epithelially expressed receptor DR5 upon IV infection remain elusive. IV (PR/8, H1N1; x31, H3N2) infection resulted in transcriptional and translational upregulation of TRAIL in murine and human primary alveolar macrophages (AM), and in enhanced expression of DR5 in isolated type I AEC. IV-induced TRAIL upregulation was dependent on protein kinase R (PKR)-mediated NF-kB activation and interferon-ß (IFN-ß) released from AM and AEC, as demonstrated by use of PKR-deficient AM and specific inhibitors. Additionally, TRAIL was upregulated on FACS-separated F4/80-positive AM in PR/8 infected C57BL/6 mice at day 8 post infection when PR/8 was largely cleared and type I IFN levels were increased in lung homogenates. Finally, abrogation of TRAIL signalling resulted in delayed IV clearance, however significantly reduced AEC apoptosis, alveolar leakage and mortality in PR/8 infected mice. These data demonstrate that IFN-ß released from PR/8-infected AM and AEC in a PKR-NF-kB-dependent manner is an important auto-/paracrine inducer of macrophage TRAIL expression significantly contributing to loss of alveolar epithelial barrier integrity during severe IV pneumonia. Future in vivo studies using gene-targeted mice will reveal the putative effect of TRAIL-inducers PKR and IFN-ß on barrier function and IV clearance.

Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes (漿樹突狀細胞的免疫原性劃定流感疫苗亞型)

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A variety of different vaccine types are available for H1N1 influenza A virus infections, however the key innate immune mechanisms controlling their immunogenicity remain unclear. Using transgenic mice, we found that plasmacytoid dendritic cells (pDC) and type-I IFN-mediated intra- and inter-cellular signalling were essential to induce a viral-specific immune response following vaccination with a killed virion. The cell surface receptor Toll-like receptor (TLR) 7, mediated immunogenicity of both live and killed virion vaccines. However, a virion-free split vaccine commonly used in humans failed to activate TLR7 and immunize naïve mice. Addition of a pDC-activating adjuvant with the split vaccine restored immunogenicity in mice. Split vaccine alone could recall memory T cell responses in human cultured cells, underscoring the importance of this pathway for priming, but not secondary immunization.

Influenza viral infection induced immune responses correlate with the pathogenesis on patients (流感病毒感染引起的免疫反應與患者的發病機制相關)

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Background: Influenza viral infection is the worldwide serious threat for human health. Therefore, understanding the immunopathogenic mechanisms and immune responses to viral infection are of critical importance to explore the better strategy for preventing the influenza viral infection.

Methods: Our study focuses on analysis of the frequency of different lymphocyte subsets, sera level of pro-inflammatory chemokines and cytokines in patients with influenza A and B viral infection. Whole blood samples and serum samples were collected from patients on enroll date and subsequently collected every 3-7 days. The results derived from samples collected on enroll date were compared with results derived from the last time point of therapy in immunological changes.

Results: The results showed that type A influenza viruses infected patients, whose total $CD4^+$ $CD8^+$ T cells were increased and the percentage of NK cells was decreased. Consistently, the varied trends of $CD4^+$ T, $CD8^+$ T cells and NK cells are revealed in patients with type B influenza virus infection as similar as in patients with type A influenza virus infection. To compare with normal group, the serum level of pro-inflammatory chemokines IL-8, RANTES, IP-10 and cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα) were increased initially during the infection and were decreased gradually in both type A and type B influenza viruses infected patients.

Conclusion: These data indicated that immune responses are activated by viral infection. Increased pro-inflammatory cytokines after influenza infection might contribute to the subsequent tissue damages in viral infection.

Infection by swine, avian and human influenza A 2009 virus and poly-IC stimulation differentially up-regulate surface markers and cytokine secretion on porcine dendritic cells in vitro (感染豬，禽和人類 2009 年的流感病毒，與聚- IC 的刺激分別上調體外豬的樹突狀細胞表面標誌和細胞激素的分泌)

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Dendritic cells (DC) link innate and adaptive immune system, expressing specialized pattern-recognition receptors (PRRs) which recognise particular pathogen-associated molecular patterns (PAMPs). Furthermore, there is growing evidence that the so-called "early" cytokines play an important role in influenza virus infection.
Our main goal was to characterize the interaction of porcine bone marrow derived dendritic cells (poBMDC) with swine, avian and human influenza virus *in vitro*.
Porcine BMDC were generated and DC morphology and virus infection evaluated by transmission electron microscopy. poBMDC were infected with A/Swine/Spain/80598-LP1/2007(H3N2), High pathogenic A/Ckicken/Italy/13474/99(H7N1), Low pathogenic A/Anas plathyrhynchos/Spain/1877/2009(H7N2), pandemic A/Catalonia/63/2009(H1N1) viruses or stimulated with TLR agonists (Poly-IC, LPS or R837). DC phenotype was analysed by flow cytometry at 16 and 24 hours, whereas IFN-α, TNF-α, IL-12 and IL-18 secretion were analysed by ELISA and IL-10 and TGF-β by RT-qPCR at 4, 8, 16 and 24 hours post infection.
Infected-poBMDC presented different phenotype by means of SLAI, SLAII and CD80/86 up-regulation. Different cytokine kinetic profile of IFN-α, TNF-α, IL-18 and IL-12 were observed depending on the virus used. Stimulation with TLR agonists induced up-regulation of SLAI, SLAII and CD80/86 and different kinetic profile in secreted cytokines, being high responders to Poly-IC and moderate responders to LPS. No induction of IL-10 and TGF-β mRNA was detected in infected cells.
The different responses observed in poBMDC infected with influenza virus or stimulated with TLR agonists pave the way for understanding the intimate relation between influenza viruses and porcine dendritic cells for triggering the mechanisms driving to protective immune response.

Analysis of long pentraxin 3 as a biomarker in pigs (分析長 pentraxin 3 作為豬生物標誌物)

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Long pentraxin 3 (PTX3) is a conserved pattern-recognition protein and a host-defense-related component of the humoral innate immune system, produced in different cell types. PTX3 has an antiviral role in early host defence against influenza infections and it might be a useful biomarker of acute lung injury in mice. Moreover, it is related to female fertility in mice.
The aim of our study was to investigate the role of PTX3 as a biomarker *in vivo* and *in vitro* in pigs.
For *in vitro* studies, porcine bone marrow-derived dendritic cells (poBMDC) were generated and infected with H3N2 swine influenza virus (SIV) (A/Swine/Spain/80598-LP1/2007). For

in vivo study, sixteen conventional pig farms with history of pleuritis lesions and cranio-ventral lung consolidation were used. Haptoglobin (Hp), C-reactive protein and Pig-MAP were determined in serum of 20 randomly selected pigs. Moreover, sera from 48 conventional sows from parity one to eight showing high SIV antibody titres were also used. PTX3 concentration in sera and culture supernatant was determined by sandwich ELISA (2C3 and 6B11 antibodies). Statistical analysis was performed by linear regression and a Kruskal-Wallis test.
The *in vitro* study showed that myeloid poBMDC produced PTX3 after infection with SIV.*In vivo* results showed that PTX3 is not a good biomarker for chronic lung lesion status in swine farms when compared to previous studies with Hp and Pig-MAP. However, PTX3 concentration in SIV antibody positive sera correlated with a greater exposure to infections, indicating that PTX3 might be used as a biomarker under certain conditions

Responses of human monocyte-derived dendritic cells to co-infection of influenza virus and *Streptococcus pneumoniae* (人類單核細胞樹突狀細胞對合併感染流感病毒和肺炎鏈球菌的反應)
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Influenza virus infection followed by subsequent bacterial stimulation often results in disease severity. In this study, we investigated the response of human monocyte-derived dendritic cells (MDDCs) to co-infection of influenza virus H1N1 and *S. pneumoniae*. The cell apoptosis, phenotype maturation, cytokine and chemokine production in H1N1 (stain: HK/54/98) virus-infected MDDCs upon heat-inactivated *S. pneumoniae* (strain: 09M4578 serotype 14) stimulation were examined. Upon *S. pneumoniae* stimulation, H1N1 virus-infected MDDCs underwent greater apoptosis and displayed lower CD86 and MHC-II expression than that of mock-infected MDDCs. Functionally, *S. pneumoniae* induced significantly synergic increases of the productions of chemokine: MIP-a, IL-8, and cytokine: TNF-a, IL-6, IL-12, IFN-g in H1N1 virus-infected MDDCs when cells were stimulated with influenza virus and *S .pneumoniae* simultaneously, as compared to mock-infected MDDCs, However, the synergy in cytokine production responses induced by secondary *S. pneumoniae* decreased when MDDCs were stimulated with *S. pneumoniae* 24hrs post primary influenza infection instead of simultaneously. In contrast, although single *S. pneumoniae* stimulation induced MDDCs to produce IL-10 in a dose dependent manner, prior exposure to influenza suppressed IL-10 production in MDDCs. Our results suggest that disease severity of post-influenza pneumococcal pneumoniae may be attributed to the decrease in viable DC numbers to control infection, and altered cytokine and chemokine patterns which may disrupt the normal T cell priming function of DC during *S.pneumoniae* stimulation.

Type I interferon signaling is required for control of monocytes and neutrophils in the lung after influenza virus infection (I 型干擾素的信號是流感病毒感染後控制肺單核細胞和中性粒細胞所必需的)
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Recent studies revealed that type I interferon (IFN-I) plays a critical role in the regulation and homeostasis of hematopoietic stem cells in addition to activation of antiviral cascades. To elucidate the role of IFN-I on the pulmonary immune responses after virus infection, we infected mice lacking a type I interferon receptor (IFNAR1) and wild-type B6 mice with

influenza A virus ($1x10^5$ pfu of A/PR8, H1N1). $IFNAR1^{-/-}$ mice had higher mortality and higher levels of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, and IFN-γ) in bronchoalveolar lavage samples than wild-type mice. Although wild-type mice were able to generate $CD11c^{low}Ly6C^{hi}Ly6G^{neg}$ foamy macrophages following influenza infection, $IFNAR1^{-/-}$ mice failed to do so. However, $IFNAR1^{-/-}$ mice generated $CD11c^{int}Ly6C^{int}Ly6G^{neg}$ heterologous monocytes. These two different $Ly6C^{pos}$ monocyte types contribute to different immune cell patterns by producing different types of chemokines. Foamy macrophages mainly produce MCP-1 and maintain their populations by recruiting monocyte precursors while the $CD11c^{int}Ly6C^{int}Ly6G^{neg}$ monocytes of $IFNAR1^{-/-}$ mice mainly produce KC, a well-known factor for neutrophil influx. As a result, neutrophil recruitment in $IFNAR1^{-/-}$ mice is significantly elevated when compared to wild-type mice and remains elevated in a time-dependent manner after influenza virus infection. When we used bone marrow (BM) chimera mice (wild-type BM into $IFNAR1^{-/-}$ and vice versa), we found that BM cells isolated from $IFNAR1^{-/-}$ mice failed to differentiate $Ly6C^{pos}$ monocytes into $Ly6C^{hi}$ cells. We suggest that the balance between monocytes and neutrophils, which are mainly regulated by IFN-I, contribute to the control of inflammation caused by virus infection.

An inactivated whole particle vaccine from a non-pathogenic virus library confers protective immunity against H1N1 pandemic influenza virus more effectively than does a split vaccine in cynomolgus macaques (從非致病性病毒庫來之滅活性完整顆粒疫苗賦予食蟹獼猴 H1N1 流感病毒保護性免疫預防比不分裂疫苗更有效)

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Development of pandemic (H1N1) 2009 influenza virus vaccines is an urgent issue since viral pneumonia in human cases has been reported and mortality caused by this virus infection seems to be higher than seasonal influenza virus infection in the early reports. We made an H1N1 vaccine candidate from an influenza virus library of non-pathogenic type A influenza viruses and examined protective effects against a pandemic (H1N1) 2009 strain using cynomolgus macaques. Antibody responses specific for the vaccine strain A/swine/Hokkaido/2/1981 (H1N1) were elicited by subcutaneous inoculation into macaques with the inactivated whole virus particle vaccine without adjuvant more vigorously than with the split vaccine. Sera of macaques immunized with the whole particle vaccine showed higher neutralization titers against the vaccine strain than did those with the split vaccine. Neutralization titers against a pandemic (H1N1) 2009 strain in sera of macaques immunized twice with the split vaccine reached a similar level of those observed in sera of macaques immunized once with the whole particle vaccine. However, after challenge with the pandemic virus, the whole particle vaccine apparently decreased viral replication in the nasal swabs of macaques, while the split vaccine did not. These findings make an assumption that the whole particle vaccine but not the ether split vaccine would induce CTL activity against the challenge strain. In any case, we concluded that the whole particle vaccine from our virus library conferred more effective protective immunity to macaques against pandemic influenza virus infection than did the split vaccine.

Immunoprotective effects of pneumococcal surface protein A (PspA) on secondary pneumococcal pneumonia after influenza virus infection（感染流感病毒後繼發肺炎球菌性肺炎，表面蛋白 A（PspA）對肺炎球菌的免疫保護作用)

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S.pneumoniae is the most common pathogen of secondary bacterial pneumonia, which can cause excess morbidity and mortality, after influenza virus infection. However, there has been no evidence that pneumococcal vaccines can protect against pneumococcal infection after influenza infection. We report here that secondary pneumococcal pneumonia can be prevented by nasal immunization of pneumococcal vaccine candidates, pneumococcal surface protein A (PspA). PspA-specific antibody was induced in mice nasally immunized with PspA, and bacterial clearance in lungs was enhanced in mice immunized with PspA. Moreover, survival rate was increased and sera from PspA-immunized mice enhanced C3 complement deposition on the bacteria. These results suggest that nasal immunization of PspA can protect against secondary pneumococcal pneumonia after influenza infection.

Zymosan increases the production of secretory IgA antibodies against influenza virus by enhancing the mucosal adjuvant activity (酵母菌瘁取物 Zymosan 透過提高粘膜佐劑活性增加抗流感病毒分泌性 IgA 抗體生產)

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Intranasal vaccination against the influenza virus could block infection by inducing cross-protective secretory IgA antibodies in the upper respiratory tract. The synthetic double-stranded RNA polyriboinocinic polyribocytidylic acid [poly(I:C)] is a potent mucosal adjuvant in mice immunized intranasally with an inactivated influenza vaccine. In this study, to increase the effectiveness of a nasal poly(I:C)-combined vaccine, the effect of zymosan, a cell wall extract from *Saccharomyces cervisiae* was investigated, on the adjuvant activity of poly(I:C).

The addition of zymosan as an adjuvant in BALB/c mice which were immunized intranasally with a poly(I:C)-combined vaccine enhanced the ability of the mice to mount an effective immune response to not only homologous virus but heterologous influenza virus, that antigenicity largely differs from the vaccinated strain. The secretory IgA and serum IgG antibody levels increased synergistically. To define the mechanism by which zymosan enhanced the adjuvant activity of poly(I:C), bone marrow-derived dendritic cells (BM-DCs) were cultured in the presence of poly(I:C) and/or zymosan. There was a synergistic increase in cytokine production in BM-DCs in response to co-treatment with poly(I:C) and zymosan. This synergistic effect on cytokine production was mimicked by co-treatment with poly(I:C) and a Toll-like receptor 2 (TLR2) ligand, which represented one of the components of zymosan. These results suggest that one of the mechanisms by which zymosan enhances the adjuvant activity of poly(I:C) is through increased cytokine production by DCs involving the synergistic activation of poly(I:C)-induced TLR3- and zymosan-induced TLR2-mediated signaling pathways.

T-cell independent activation of virus-specific memory B cells requires Toll-like receptor (TLR) signaling(需要 Toll 樣受體（TLR）信號活化 T 細胞獨立性病毒特異性記憶 B 細胞)

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Memory B cells are generated by infectious pathogens and confer protection against the re-infection of the same pathogens. Recent findings have shown one of the remarkable features of memory B cells elicited by infectious pathogen, which is T-cell independent activation by antigen re-exposure. However the mechanisms underlying these processes remain unknown. Here, we have analyzed hemagglutinin (HA)-specific memory B cells generated by intranasal infection with influenza virus. Adoptive transfer experiments revealed that class-switched memory B cells with B-2 phenotype responded to virus particles, but not to soluble HA protein of influenza virus, in the absence of T cell help. Immunization with inactivated virus particles also generated memory B cells that could be reactivated without T cells, while immunization with soluble HA protein failed. These results indicate that the viral component rather than viral replication is required for the generation of those memory B cells. To address how memory B cells are reactivated in the absence of T cell help, we utilized MyD88/TRIF double deficient mice as a source of memory B cells. We observed that memory B cells of the double deficient mice could not respond to virus particles in the absence of T cell help, showing that TLR signals are essential for the generation and/or reactivation of the memory B cells. These results give new insights into the cellular and molecular basis for memory B cell responses elicited by infectious pathogens, such as influenza virus.

Immune responses to H1N1 pandemic influenza in a longitudinal cohort study (H1N1 流感大流行的免疫反應縱世代研究)

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Human immune responses to the recent H1N1 pandemic influenza may provide information on the role that heterosubtypic immunity might play in protection from transmission or ameliorating disease severity. This longitudinal cohort study assessed immune responses to H1N1, H3N2, and B strains in these subject groups: H1N1 pandemic influenza-infected; those presenting with influenza-like illness but influenza virus negative; and healthy donors. In addition we also assessed responses in individuals vaccinated with the H1N1 pandemic influenza vaccine (Panvax) or the 2009 seasonal vaccine. Antibody responses were tested in haemagglutination inhibition (HI), and microneutralisation (MN) assays. Influenza-specific memory B cell responses were also assessed by ELISpot. HI titres to H1N1 pandemic influenza were high in the majority of those infected with H1N1 pandemic influenza, while some individuals not confirmed to be infected with H1N1 pandemic influenza also had high H1N1 pandemic HI titres at enrolment, indicating possible recent subclinical exposure, or that some individuals have existing heterosubtypic antibody responses to the H1N1 pandemic strain. H1N1 pandemic influenza-infected individuals demonstrated increasing frequencies of H1N1 pandemic-specific IgG-secreting B cells from 0.02% at Day 1, to up to a peak of 11% at Day 14. Frequencies of IgG-secreting B cells specific for older circulating strains of H1N1

virus also increased. Individuals who received Panvax also had an increase in H1N1 pandemic-specific IgG-secreting B cells at Day 14, however, some had a detectable increase only to an older H1N1 strain, indicating that memory immune responses and 'original antigenic sin' may influence the nature of the antibody response.

Host defense against influenza (H5N1 and H1N1) infection (宿主對抗流感（H5N1 和 H1N1 病毒）感染)

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Risk parameters of avian influenza (H5N1) [J. Infectious Dis., 2009]: The H5N1-positive patients had ARDS with normal ventilation capacity at the time of hospital admission in National Hospital of Pediatrics (NHP, Hanoi, Vietnam), then rapidly proceeded to severe respiratory failure. The survival probabilities in groups of H5N1-positive vs. H5N1-negative patients were 17% versus 52% with clinical picture of H5N1-induced fulminant ARDS.
Model mice for FARDS: We inoculated PR8-H1N1 into mice, and then cytokine-chemokine levels in plasma, bronchoalveolar lavage fluid were analyzed. Specific chemokines were induced with infiltration of neutrophils. In addition, influenza-induced pneumonia in MPO-KO mice developed less than that in wild-type mice, suggesting that MPO released from infiltrated neutrophils may induce lung injury.
Measures against pandemic H1N1 influenza [Eurosurveillance, 2009]: Modelling methods are needed to estimate the validity of these measures before their implementation on a large scale. We showed that post-exposure prophylaxis combined with isolation at home and school closure significantly decreases the total number of cases in the community and can mitigate the spread of pandemic H1N1 influenza, even when there is a delay in the availability of vaccine.

Immunogenic potential of a virosome-based avian influenza vaccine (virosome 爲基礎的禽流感疫苗具有潛在的免疫原性)

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Several types of vaccines are currently available for control of avian influenza virus (AIV), including inactivated influenza virus-vectored recombinant as well as subunit vaccines. However, subunit vaccines are generally poorly immunogenic, compared to conventional vaccines, unless they are administered using suitable delivery vehicles or adjuvants. In the present work we have evaluated immunogenicity of fusion-active virosomes for elicitation of immune responses. Furthermore, we asked whether combining interferon-gamma (IFN-γ) or CpG ODN with virosomes would enhance immunogenicity of virosomes. Virosomes prepared from H4N6 avian influenza virus were used as an immunogen alone or in combination with purified baculovirus expressed recombinant chicken (rch) IFN-γ or CpG ODN. Using a prime-boost regimen, birds were immunized with various vaccine formulations and sera were collected on a weekly basis to measure antibody responses. In addition, spleens were collected for measuring T cell responses. All birds immunized with various virosome preparations were seroconverted. Moreover, combining CpG ODN with virosomes

significantly increased haemagglutinin inhibition (HI) antibody titers compared to birds those were immunized with virosomes alone or virosomes with rchIFN-γ. Immunization with virosomes combined with CpG elicited the highest amount of serum IgG and IgA against the whole virus, followed by immunization with virosomes plus rchIFN-γ, and virosomes alone. Furthermore, we noted higher cellular activation in the group that had received virosomes +CpG ODN compared to the groups that received virosome + IFN-γ or virosomes alone. In conclusion, our results demonstrate that immune responses to virosome formulation against influenza virus can be further enhanced by CpG ODN.

Chicken IFN-lambda as an antiviral therapeutic (雞干擾素λ作爲抗病毒治療)

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Interferons (IFN) provide a critical first line of defence against viral infection in vertebrates. Moreover, IFNλ, a recently identified group of mammalian IFN, has demonstrated antiviral potential in the treatment of mammalian viruses. With the growing concern over diseases, such as avian influenza, there is a pressing need for new antiviral strategies to manage problem viruses in poultry. Furthermore, the use of immune molecules, such as IFNλ, provides an attractive option for treating poultry by augmenting the host response to virus. With this in mind, we cloned and expressed the chicken orthologue of IFNλ (ChIFNλ) and assessed the biological activity and anti-viral potential of this avian cytokine. Analysis of the expressed recombinant ChIFNλ showed that, similar to the observations with mammalian IFNλ, ChIFNλ has viral inhibitory properties similar to that observed for type 1 IFN. However, in the assays used ChIFNλ appeared to exhibit these activities at a lower level. Additionally, although ChIFNλ did show activity similar to type 1 IFN, ChIFNλ could be distinguished from type 1 IFN as it had dissimilar induction properties and was associated with the induction of different genes. These observations might suggest that although ChIFNλ has properties similar to type 1 IFN, ChIFNλ may have a differential role in response to virus. Consequently, the discovery of ChIFNλ, and its identified activity provides an opportunity for alternative IFN therapy in viral management. The observed antiviral activity demonstrated by ChIFNλ supports its potential inclusion in therapeutic strategies directed against viral infections.

Responses of human monocyte-derived dendritic cells to co-infection of influenza virus and *Streptococcus pneumoniae* (人單核細胞衍生之樹突狀細胞對合併感染流感病毒和肺炎鏈球菌的反應)

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Influenza virus infection followed by subsequent bacterial stimulation often results in disease severity. In this study, we investigated the response of human monocyte-derived dendritic cells (MDDCs) to co-infection of influenza virus H1N1 and *S. pneumoniae*. The cell apoptosis, phenotype maturation, cytokine and chemokine production in H1N1 (stain: HK/54/98) virus-infected MDDCs upon heat-inactivated *S. pneumoniae* (strain: 09M4578 serotype 14) stimulation were examined. Upon *S. pneumoniae* stimulation, H1N1 virus-infected MDDCs underwent greater apoptosis and displayed lower CD86 and MHC-II expression than that of mock-infected MDDCs. Functionally, *S. pneumoniae* induced significantly synergic increases of the productions of chemokine: MIP-a, IL-8, and cytokine:

TNF-a, IL-6, IL-12, IFN-g in H1N1 virus-infected MDDCs when cells were stimulated with influenza virus and *S .pneumoniae* simultaneously, as compared to mock-infected MDDCs, However, the synergy in cytokine production responses induced by secondary *S. pneumoniae* decreased when MDDCs were stimulated with *S. pneumoniae* 24hrs post primary influenza infection instead of simultaneously. In contrast, although single *S. pneumoniae* stimulation induced MDDCs to produce IL-10 in a dose dependent manner, prior exposure to influenza suppressed IL-10 production in MDDCs. Our results suggest that disease severity of post-influenza pneumococcal pneumoniae may be attributed to the decrease in viable DC numbers to control infection, and altered cytokine and chemokine patterns which may disrupt the normal T cell priming function of DC during *S.pneumoniae* stimulation.

Type I interferon signaling is required for control of monocytes and neutrophils in the lung after influenza virus infection (I 型干擾素的信號是流感病毒感染後控制在肺單核細胞和中性粒細胞所必需的)

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Recent studies revealed that type I interferon (IFN-I) plays a critical role in the regulation and homeostasis of hematopoietic stem cells in addition to activation of antiviral cascades. To elucidate the role of IFN-I on the pulmonary immune responses after virus infection, we infected mice lacking a type I interferon receptor (IFNAR1) and wild-type B6 mice with influenza A virus ($1x10^5$ pfu of A/PR8, H1N1). $IFNAR1^{-/-}$ mice had higher mortality and higher levels of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, and IFN-γ) in bronchoalveolar lavage samples than wild-type mice. Although wild-type mice were able to generate $CD11c^{low}Ly6C^{hi}Ly6G^{neg}$ foamy macrophages following influenza infection, $IFNAR1^{-/-}$ mice failed to do so. However, $IFNAR1^{-/-}$ mice generated $CD11c^{int}Ly6C^{int}Ly6G^{neg}$ heterologous monocytes. These two different $Ly6C^{pos}$ monocyte types contribute to different immune cell patterns by producing different types of chemokines. Foamy macrophages mainly produce MCP-1 and maintain their populations by recruiting monocyte precursors while the $CD11c^{int}Ly6C^{int}Ly6G^{neg}$ monocytes of $IFNAR1^{-/-}$ mice mainly produce KC, a well-known factor for neutrophil influx. As a result, neutrophil recruitment in $IFNAR1^{-/-}$ mice is significantly elevated when compared to wild-type mice and remains elevated in a time-dependent manner after influenza virus infection. When we used bone marrow (BM) chimera mice (wild-type BM into $IFNAR1^{-/-}$ and vice versa), we found that BM cells isolated from $IFNAR1^{-/-}$ mice failed to differentiate $Ly6C^{pos}$ monocytes into $Ly6C^{hi}$ cells. We suggest that the balance between monocytes and neutrophils, which are mainly regulated by IFN-I, contribute to the control of inflammation caused by virus infection.

The role of IL-1 signaling in lung tissue recovery from influenza-induced damage (流感引起肺組織損傷需要 IL - 1 信號進行修復)

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Components of the NLRP3 inflammasome are critical for survival to highly pathogenic influenza infection. As NLRP3−/− and Caspase-1−/− mice die late in the infection process_days 10-13 – with normal adaptive immune functions, it is possible that the dysregulated lung tissue repair response and subsequent systemic hypoxia found at this stage

in these mice may have caused death. We propose that the immediate caspase-1 substrates IL-1β and IL-18 are required for an appropriate lung tissue healing response for recovery from influenza infection. We have found PR/8-treated IL-1R1−/− mice to be more prone to death, and viral loads at days 3, 6, and 9-11 are similar to those of WT mice, suggesting IL-1R1−/− mice clear virus appropriately. Thus, IL-1 signaling appears to be necessary for survival to influenza infection. We have characterized blood oxygenation levels in IL-1R1−/− and IL-18−/− mice in response to PR/8 infection and studies to determine the mechanisms of respiratory epithelial layer damage and repair using in vitro and in vivo model systems are underway.
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Human influenza elicited an effective antiviral suppression than avian influenza in bronchial epithelial cells (人類流感病毒較禽流感病毒能有效引起支氣管上皮細胞的抗病毒抑制作用)
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Background: Human bronchial epithelial cells (BECs) are the primary site of infection by influenza viruses, and yet they were unable to respond to human influenza as efficiently as they would to low pathogenic avian influenza. Our aim was to examine the underlying cause for this difference in anti-viral response.
Methods: Human A/H3N2 and a low pathogenic avian influenza A/H11N9/Sandpiper were used to infect Calu-3 cells at an MOI of 5. Supernatants were harvested for interferon (IFN)-β protein detection by western blotting, CXCL-10 and IFN-λ1 was measured by ELISA. Viral replication was analysed by plaque assay on MDCK cells. NS1 genes of H3N2 and H11N9 was cloned and transfected into Calu-3. Following Poly I:C stimulation, the same measurements were performed. Whole cell lysates were collected for NS1 expression by western blotting. Total RNA was collected at 24hr after infection for microarray gene expression analysis.
Results: Human influenza induced a delayed RIG-I signalling and lower IFN-β, IFN-λ1, and CXCL-10 protein expression in Calu-3 compared to avian influenza, leading to a higher H3N2 replication. Transfection of H3N2 NS1 showed greater inhibition of these anti-viral proteins in Calu-3 than H11N9 NS1 did. This potent suppression was also observed across the entire anti-viral-associated genes.
Conclusion: Human influenza replicated more effectively in BECs compared to avian influenza, this was due to more effective anti-viral suppression by human influenza NS-1 gene. Adaption of the NS-1 gene is an important factor in promoting infection of human BECs.

Characterization of lung dendritic cell function using lipophilic dye-labelled influenza virus (使用脂溶性染料標記的流感病毒研究肺部樹突狀細胞功能)
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Lung dendritic cells (DCs) are central to the initiation of the immune response to influenza A infection. However, the relative contribution of different lung DC populations to viral uptake, transport and CD8 T-cell priming remain poorly understood. To address this, we labelled influenza virus with fluorescent lipophilic dye to visualise uptake of the virus as well as to

map the primary immune response during the early stages of infection. We show that CD11blo/negCD103+ DCs rapidly transport influenza to the posterior mediastinal lymph node where they are responsible for initiating virus-specific CD8 T-cell replication. In contrast, CD11bhiCD103- DCs remain in the lung to take up virus and release inflammatory cytokines. This demonstrates that lung DCs have functionally different responses to influenza and has implications for DC-targeted antigen delivery vaccine strategies aimed at generating cross-protective CD8 T-cell immunity against influenza.

Characterization of protective immune response of Swine-Origin Influenza Virus (S-OIV) H1N1 vaccine on mice (豬源流感病毒（S-OIV）疫苗對小鼠保護性免疫反應研究)

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The novel influenza A (S-OIV H1N1) virus emerged in 2009 has causing the World Health Organization to declare an emergent pandemic. Proper vaccination is still the practical method to prevent the epidemic of influenza within community. In this study, NIBRG-121 vaccine was characterized for its immune response and protection against lethal challenge of A/California/7/2009 H1N1 virus. First, immunization dose and the lowest virus titer of attenuated vaccine virus were investigated for the protection of BALB/c mice. Mice immunized with one or two dose of attenuated virus accompanied with aluminum hydroxide or MPLA adjuvant were challenged with 10^6 $TCID_{50}$ of A/California/7/2009 H1N1 virus. Results showed that single dose immunization of NIBRG-121 (10^3 $TCID_{50}$) sufficiently protected mice from lethal challenge of S-OIV H1N1 virus. Second, the lowest protective dose and immune response of S-OIV H1N1 vaccine was evaluated. Mice immunized with single dose of 0.05 μg S-OIV H1N1 vaccine were resistant to the lethal challenge of wild-type virus (85.7% survival). The minimum effective doses of single dose immunization were reduced to 0.01 μg and 0.001 μg in the presence of Alum (100% survival) and MPLA (100% survival) adjuvant, respectively. The body weight loss was also less significant for mice immunized with vaccine plus adjuvant than mice immunized with vaccine only. Further, MPLA adjuvant had better effect than aluminum hydroxide, especially in the induction of Th1-type antibody response (IgG.2a). Results from this study implicated that single dose (15 μg HA) of NIBRG-121 vaccine should be sufficient to protect human from infection of S-OIV H1N1 virus.

Surveying the Influenza-specific cytotoxic T cell response in humans and mice using combinatorial tetramer staining and mass-cytometry (CyTOF) (組合四聚體染色和質譜儀對人類和小鼠的流感特異性細胞毒性 T 細胞反應進行研究)

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The direct detection of antigen-specific T cells using tetramers of soluble peptide-major histocompatibilty complex (pMHC) molecules is widely used in both basic and clinical immunology. However, the number of specificities that can be assessed simultaneously has been a major limitation. We recently described and validated a method using combinations of fluorescent pMHC tetramers to simultaneously detect large numbers (>15) of T cell specificities in a single human blood sample. We are now extending this method using a new form of flow cytometry that uses heavy metal labels and single-cell time-of-flight mass spectroscopy instead of fluorophores (a.k.a., Cytometry via Time of Flight - CyTOF). CyTOF

has several benefits over traditional fluorescence-based flow cytometry, including the ability to detect >30 different tags per cell without the need for any compensation for spectral overlap. Here we have applied these novel methods to assess the breadth of the antigen specific cytotoxic T cell response to a wide array of Influenza epitopes.

Increased frequency of CCR5delta32 allele among individuals admitted to intensive care units with severe respiratory illness caused by Pandemic A/H1N1 influenza (甲型 H1N1 流感大流行引起的住進加護病房重症呼吸疾病個體 CCR5δ32 等位基因頻率增加)

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Background: The recent H1N1 influenza pandemic caused symptoms with a wide range of severity. Although a number of risk factors for severe symptoms have been identified, the immunological mechanisms contributing to H1N1 pathogenesis are not fully understood. Recently, the CCR5delta32 polymorphism (which prevents the expression of functional CCR5 receptor) was associated with increased risk of severe West Nile neurological symptoms. The CCR5 receptor is also implicated in control of influenza infection in mice. This study therefore aims to determine whether the CCR5delta32 polymorphism is associated with severe H1N1 respiratory illness.
Methods: DNA was extracted from lymphocytes obtained from 19 patients who were hospitalized for severe respiratory illness caused by confirmed H1N1 pandemic influenza. The presence of the CCR5delta32 allele was determined by PCR amplification of the delta32 locus and the size of the resulting PCR product.
Results: 10/19 patients were of Aboriginal ethnicity and, as expected, did not carry the CCR5delta32 allele. Of the 9 Caucasian patients, 5 were heterozygous for the allele, giving an allele frequency of 27.8%, compared to the average frequency of 10% among Caucasian North American populations.
Conclusions: The CCR5delta32 allele was highly enriched in Caucaisan patients hospitalized for severe H1N1 infection, suggesting that CCR5 genotype is a risk factor for severe infection and implicating CCR5 in the control of H1N1 infection. This is consistent with the role CCR5 plays in trafficking of lymphocytes to the lungs in animal models of influenza infection. Future studies will address the impact of other immune polymorphisms in these patients.

Characterization of human anti-influenza M2 antibody obtained from peripheral blood of vaccinated volunteer (疫苗志願接種者周邊血獲得的人抗流感 M2 抗體特性研究)

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In immune response, highly diversified B-cell repertoires are generated to protect human body against pathogenic microorganisms. To detect and analyze human antigen (Ag)-specific B-cells in peripheral blood, we previously reported a novel screening method, ISAAC. In this study, we applied this method to detect influenza virus-specific B-cells in peripheral blood from healthy volunteers who had been immunized with influenza HA vaccine to generate human influenza HA monoclonal antibodies. We obtained many human monoclonal antibodies reacting influenza viruses, and characterized their functions. Surprisingly, one of them specifically bound external domain of the matrix protein 2 (M2e), that is highly conserved between influenza virus strains compared to haemagglutinin and neuraminidase, and has been long prospected as a potential universal vaccine target. It has been believed that detection of M2e-specific antibody producing B-cells from human peripheral blood

lymphocytes is difficult, since M2e induces no or only a weak and transient immune responses following infection. We demonstrated that the M2e-specific antibody we obtained in fact reacted to influenza A viruses, including 2009 H1N1 pandemics and highly pathogenic avian influenza A virus. More importantly, it inhibited viral replication in vitro, suggesting that it could be a potent candidate for medical application. In conclusion, we could obtain an influenza virus M2e-specific human monoclonal antibody from a vaccinated human volunteer using ISAAC method. ISAAC is expected to be a powerful method to obtain human monoclonal antibodies against a weak antigenic epitopes of pathogenic microorganisms directly from infected patients or immunized volunteers.

The generation of transbody specific to viral matrix protein 1 (M1) for influenza therapy (產生對病毒基質蛋白 1(M1)的 transbody 以作為流感的治療)

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Influenza virus causes epidemic and pandemic worldwide in the history yielding both economic and social lost. Not only livestock, but mammals including human also served as infectious host for the virus. Anti-viral agents and vaccines faced the limitations about the viral antigenic variation of the surface molecules. Thus, internal conserved proteins might be the interesting targets for influenza virus therapy. Among 11 influenza virus proteins, matrix protein 1 (M1) is abundant and highly conserved. It plays many pivotal roles during viral replication cycles including uncoating, nuclear import and export, and virion assembly. Blocking M1 protein is supposed to limit the viral replication in the infected individual.
In this study, Cell-penetrating peptide (CPP), namely Penetratin (PEN),-coding sequence was generated by PCR method and molecularly linked to M1-specific HuScFv-coding sequence to create PEN-HuScFv (transbody) DNA construct. The fully human monoclonal PEN-HuScFv was produced and purified from bacteria. It could exhibit membrane translocation across MDCK cells with intact cellular membrane and also penetrate membrane of influenza virus-infected MDCK cells to target cytoplasmic viral M1. The effect of PEN-HuScFv on influenza virus replication was determined in infected MDCK cell monolayers by RT-PCR and plaque formation assay. The production of newly formed infectious virion was diminished upon incubation of infected cells with PEN-HuScFv compared to controls. The results suggested that the development of viral protein-specific HuScFv with cell-penetrating activity could stop the disease-producing ability of influenza virus. PEN-HuScFv might be potential in influenza therapy.
Acknowledgement: TRF and NASTDA, Thailand

Sublingual vaccination with live influenza virus induces long-lasting protective immunity (舌下接種流感活病毒誘導持久保護性免疫)

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Our previous study showed that sublingual (s.l.) mucosa is an effective and safe vaccination route for protecting mice from lethal intranasal (i.n.) challenge with A/PR/8/34 (PR8, H1N1) influenza virus. In this study, we attempted to clarify the long-lasting protective immunity induced by s.l. vaccination. Groups of mice were immunized with live PR8 virus ($10xLD_{50}$) via the s.l. route or with formalin-inactivated PR8 virus plus cholera toxin as mucosal adjuvant via i.n. or s.l. routes. At 6 months after final vaccination, when mice were challenged

with a lethal dose of PR8 virus, the live PR8-vaccinated mice showed much lower morbidity than those given inactivated PR8-vaccine via either the i.n. or s.l. routes. When we compared the outcomes of mice given a single dose of live PR8 virus with those given three doses of inactivated PR8 virus, mice given a single dose of live PR8 virus still showed much lower morbidity than those given three doses of inactivated PR8 vaccine. Further, live PR8-vaccinated mice showed better protection against lethal heterosubtypic challenge with A/Aquatic Bird/Korea/W81/2005 (W81, H5N2) influenza virus at 6 months after s.l. vaccination. We further investigated the efficacy of X-31 cold adaptive vaccine strain, which is applicable for human use. Of note, s.l. vaccination with heterosubtypic (X-31ca-H3N2) or homosubtypic (X-31ca-H1N1) strains protected mice from PR8 (H1N1) lethal challenge. Thus, we suggest s.l. vaccination with a live form of vaccine as a long-term effective vaccination method for protection against lethal influenza virus infection.

Memory potential is determined by priming epitope number (記憶潛力是由抗原表位的數目決定)

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Immunization with an engineered H1N1 influenza A virus disrupted for two dominant CD8+ T cell epitopes led to substantially increased subdominant responses following respiratory challenge with the comparable “knockout” or wildtype H3N2 virus. Conversely, challenge with the knockout virus following wild type priming did not result in similar enhanced subdominant expansion. The basis of this compensatory effect is thus established at the time of primary infection, though there were no obvious differences in memory T cell numbers prior to secondary virus challenge or in the kinetics and magnitude of subdominant antigen presentation. The enhanced recall responses were, however, modified in both breadth and character. Single cell analysis of subdominant TCR CDR3β regions showed greater evidence of sharing between knockout-primed mice indicative of broader memory repertoire recruitment, in contrast to the more “private” response characteristic of the wildtype infection. The expanded subdominant CD8+ T cell populations in the knockout-primed mice also had a lower overall TCR avidity and persisted into secondary memory compartment. Thus, priming in the context of fewer epitopes recruits a broad repertoire with increased memory potential, suggesting possibilities for vaccination protocols skewed towards minor epitopes that might, for example, be less susceptible to immune escape.

Development and differentiation of memory CD8+ T cells in influenza infection (在感染流感後記憶 CD8＋T 細胞的發展和分化)

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Many questions still linger regarding the development and differentiation of memory CD8+ T cells during influenza infection. Previously, it has been shown that an inverse relationship exists between virus inoculum size and the CD8+ T cell response. To this end, we examined the development and differentiation of CD8+ T cell memory populations based on specificity and effector function in a mouse model system comparing high and low pathological infections of influenza. Our findings reveal that viral titer was not the determining factor in the outcome of disease. Instead, differences in CD8+ T cell inflammation and effector

function were observed for each infection dose, and these differences correlated with disease outcome. These phenotypic and functional analyses have revealed distinct effector memory CD8+ T cell populations in the context of influenza infection.
This work was supported by NIH grant RO170251

Participation of Bruton's tyrosine kinase in immune response to nasal influenza vaccination (布魯頓的酪氨酸激酶參與鼻腔接種流感疫苗的免疫反應)
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Introduction: Recently nasal administration of influenza vaccine has been investigated intensively not only because this method does not require medical manipulation but also because nasal immunization induces cross-protection to subtypes of influenza virus by production of IgA. However mechanisms underlying formation of the cross-protection remain obscure. Natural antibody is immunoglobulin produced by B-1 cells prior to infection or immunization and found to have neutralizing activity to wide range of pathogens, including all strains of influenza viruses. Therefore, we thought B-1 cells and natural antibodies might participate in the nasal vaccine-induced cross-protection. To see if B-1 cell and natural antibody is required for nasal immune response, we checked Bruton's tyrosine kinase (Btk) knockout mice, which lack B-1 cells and natural antibody, for their responsiveness to nasal influenza vaccination.
Results: In contrast to Btk+/Y controls, Btk-/Y mice were unresponsive to intra-nasally administrated influenza HA vaccine with poly(I:C) adjuvant regarding production of anti-HA IgA in nasal wash and anti-HA IgG in sera. On the other hand, Btk-/Y mice generated serum anti-HA IgG after subcutaneous administration of HA vaccine with alum adjuvant.
Discussion: From these observations, we conclude that Btk is indispensable for nasal immune response at least in this protocol but not for subcutaneous immunization. As far as our knowledge this is the first example of participation of Btk in mucosal immunity. Further investigation of specific role of Btk in nasal immune response, especially in induction of cross-protection, will reveal possible target of novel nasal vaccine adjuvant.

Added advantages using plant-based vaccine strategies against AIDS and Influenza: better immunogenicity, more heat-stable and rapid transient protein production (利用植物為基礎的流感及艾滋病疫苗防治策略優點：較好的免疫原性，更耐熱，穩定，快速的瞬態蛋白生產)
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Introduction: Plant-based vaccines have grown to be an ideal method for vaccine production, however, previous attempts to make plant-based vaccines against both AIDS and Influenza have, thus far, failed to achieve ideal immunogenic responses and swift antigen production, respectively. In this study, we expressed HIV-1 Tat and H1N1 HA-epitopes in tomato concurrently establishing better immunogenicity and highlighting the added advantage of plant-based production.
Methods: Plant-optimized gene constructs were introduced into tomato plant through bombardment and transgenic tomato lines were tested for transgene expression while observing the phenotype. Tomato extracts were introduced intradermally to Balb/c mice and immunogenic responses were observed through ELISA and ELISPOT. Tat motifs functioning

in tomato were determined through enzyme analysis and mutagenesis. Similarly, the potential uses of these motifs were explored.
Results: Preferential fusion protein expression was observed in all transgenic tomato lines and is found capable to induced both humoral and cellular immune responses in Balb/c mice. Interestingly, we found the RGD motif functioning in tomato and allow rapid protein production at relatively high amounts in just two weeks. In addition, we found that tomato cytokinin oxidase is affected through the Arg-rich motif of Tat. Ironically, the Arg-rich motif was also found to be indirectly involved in heat-stable transient protein production.
Conclusion: Expression of either HIV-1 Tat or H1N1 HA-epitopes in tomato can induce ideal immunogenic responses. Furthermore, Tat expression in tomato showed that the Arg-rich and RGD motifs of Tat function in tomato resulting to novel discoveries with the potential for further vaccine applications

Development of a T-cell inducing pan-influenza vaccine (研製 T -細胞誘導的泛流感疫苗)
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The development of an influenza ('flu) vaccine efficacious against all strains and subtypes represents a critical opportunity to reduce associated morbidity and mortality across the world. Current vaccination strategies focus on inducing neutralizing antibodies against the surface hemagglutinin protein. However, as this protein varies significantly between 'flu strains, a new seasonal vaccine has to be formulated yearly, dependent on international surveillance and estimations about prevalent circulating 'flu strains.
To circumvent these limitations, we are developing a universal T cell inducing influenza vaccine reactive toward the highly conserved internal 'flu proteins, NP & M1. Already proceeding through Phase I and Phase IIa clinical trials, this vaccine has the potential to provide protection against seasonal human influenza (H1N1 & H3N2), swine 'flu and any potential H5N1 epidemic.
Current work focuses on the delineation of the protective immune responses following vaccination and the optimisation of the regime using two immunopotent viral vectors; human adenoviruses (Ad) and Modified Vaccinia virus Ankara (MVA). By vaccinating with these MVA and Ad viruses, expressing NP and M1, we induce a potent $CD8^+$ T-cell response. Understanding how these T-cell responses are generated, and manipulating their course, is critical to the optimisation of an efficacious pan-vaccine.

A virus-like particle (VLP) vaccine for pandemic influenza A (H1N1) is safe and highly immunogenic in humans: Phase 2 clinical study (大流行流感（H1N1 病毒）病毒樣顆粒（VLP）疫苗對人類是安全的且具高度免疫原性：第 2 期臨床試驗研究)
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Influenza virus A(H1N1) 2009 caused the first pandemic outbreak of the 21st century which epicentre was Mexico. Vaccines were rapidly generated using mainly chicken egg technology. However, only 10 million doses of these vaccines were promised for Mexico who's population is over 112 million. We searched for new and safe technology to promptly reduce vaccine shortage. A VLP vaccine was selected since is rapidly manufactured using genetically engineered baculoviruses to express influenza hemagglutinin, neuraminidase, and matrix1 proteins in Sf9 insect cells. These proteins self-assemble into VLPs that resemble influenza enveloped nucleocapsids maintaining the conformationally-dependent epitopes in a non-infectious form. A phase 2 randomized double-blind, placebo-controlled study to evaluate safety, tolerability and immunogenicity of 2 immunizations with 3 dose levels (5,15 and 45 micrograms) of the VLP vaccine was performed in healthy volunteers (age 18-64). 1016 volunteers received vaccines doses and placebo. Preimmune and day 14, 22 and 36 postvaccination antibody titres were measured in sera using hemagglutination inhibition assay. Vaccine was well tolerated and safe, no serious adverse events were observed. Local and systemic solicited events were similar between placebo and vaccinated groups. All doses were immunogenic since day 14 postvaccination. 1 dose of 15g induced seroconversion (%>4-fold rise) and seroprotection (%>1:40) (95%CI) of 7% and 49% for placebo and 64% and 93% for vaccinated groups respectively. Safety was confirmed in additional 1500 volunteers. We conclude that 1 dose of 15g-VLP vaccine is safe, well tolerated and highly immunogenic in humans and can be used for extended vaccine programs

Treatment with specific IgY controls seasonal and pandemic influenza viruses without interfering with the development of adaptive immunity (以特異性 IgY 治療可控制季節性和大流行性流感病毒且不會干擾適應性免疫的發展)

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Present strategies to deal with the threat of the flu pandemic include the quarantine of infected people and animals, the use of antiviral drugs as neuraminidase and ion channel inhibitors, and vaccines developed by pharmaceutical companies under government contracts. These approaches have met up with difficulties and we aim to develop a new form of oral immunotherapy using chicken IgY antibodies. Such antibodies are easy and cheap to produce, are well tolerated in humans, and one can utilize commercial laying hens that are available globally.

Laying hens immunized with H1N1, H3N2 or H5N1 inactivated flu viruses mount a strong immune response, and IgY purified from egg yolks had high titers against the homologous viral HA antigen. Intranasal administration of IgY anti- H5N1 to mice protected against lethal infection of highly pathogenic H5N1 in 100% of the animals if administered at the same time as or one hour prior to challenge. Interestingly, IgY against H5N1 also blocked viral invasion by H1N1(PR8) during *in vitro* and *in vivo* challenges, demonstrating that IgY to H5N1 can indeed cross protect against infection with H1N1. Furthermore, mice protected by intranasal administration of influenza-specific IgY antibodies still developed protective immunological memory to the virus infection measured by serum antibodies and T cell responses.

Thus IgY anti-flu can be used to control influenza viral infection without interfering with the development of adaptive immunological memory and the heterogeneity of the IgY response to viruses in chickens generates antibodies with broadly protective activity against influenza viruses.

Plant HSP70 as carrier for the delivery of plant-expressed recombinant antigens (植物熱休克蛋白 70 作為植物表達的重組抗原的傳遞載體)

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Mammalian Heat Shock Protein 70 (HSP70) has potent immune-stimulatory properties due to its natural capability to associate with polypeptides and to bind receptors expressed on cells involved in antigen presentation (i.e. dendritic cells, macrophages and peripheral blood monocytes). The delivery to mice of HSP70-peptide complexes (purified from infected/tumor tissues or loaded *in vitro* with synthetic peptides) is able to induce the activation of innate immunity and strong peptide-specific immune responses without the need of adjuvant. The present study was aimed to explore whether plant HSP70 share similar properties with the mammalian counterpart. The analysis started by modeling the plant protein and defining the conditions that ensure maximal expression levels and optimal recovery from plant tissues. HSP70 was purified from *Nicotiana benthamiana* leaves transiently expressing an antigenic protein (Influenza A Nucleoprotein). The purification was carried out taking care of avoiding the release from HSP70 of the polypeptides chaperoned within plant cells. The subcutaneous delivery of the purified HSP70 to mice of different haplotype demonstrated that it is highly effective in priming both humoral and cell-mediated immune responses specific to the plant expressed recombinant antigen. Overall results indicate that plant-derived HSP70 shares structural and functional properties with the mammalian homologue. Natural immune responses are generally focused on few epitopes for both B and T cells and it is not always easy to identify protective epitopes. This study paves the way of using vaccine formulations based on HSP70 derived from plants expressing recombinant protein antigens to address the issue of epitope identification.

Immunostimulating effect of Mekabu-fucoidan (derived from brown alga *Undaria pinnatifida sporophylls*-mekabu-): evaluation on the basis of antibody producibility against influenza vaccine in the elderly (Mekabu 褐藻多醣硫酸酯的免疫刺激作用:依中老年人可產生抗流感疫苗抗體作評估)

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The elderly with decreased antibody producibility are known to be less responsive to vaccination. Since basic studies in vivo indicated Mekabu-fucoidan, prepared from edible traditional sea weed in Japan had immunostimulating effect, a double-blind randomized placebo-controlled study was conducted in the elderly to evaluate the immunostimulating effect.

Sixty seven subjects, 6 male and 61 female residents aged 67to 102 of our special nursing home were randomized into 2 groups who were administered 300mg Mekabu-fucoidan or fibersol-2, a kind of dietary fiber as placebo once daily during 1 month period after informed consent was obtained.

They were inoculated against Influenza HA (with A-Brisbane H1N1, Uruguay H3N2, and B-Brisbane2008) after 1 month administration of Mekabu-fucoidan or placebo, and were checked for influenza HI antibody titers 1 month after the inoculation for the comparison with the titers before the administration of Mekabu-fucoidan or placebo.

The Mekabu-fucoidan group showed greater rise in antibody titer than placebo after the

inoculation of 3 types of vaccine, and inferential statistical analysis demonstrated significant elevation of antibody titers against B-Brisbane2008 ($P<0.05$).
The efficacy of the influenza vaccine was evaluated in accordance with the efficacy parameters set by European Medicines Agency, and change rate in the titers of B-Brisbane2008 in the Mekabu-fucoidan administered group met the efficacy parameter, demonstrating the effectiveness of Mekabu-fucoidan as an immunoenhancer, while no sufficient antibody producibility was observed in placebo group. This study indicates that Mekabu-fucoidan administration can improve the efficacy of influenza vaccine in the elderly.

Evaluation and optimization of a GPI-0100 adjuvanted influenza subunit vaccine in mice (在小鼠評估並優化 GPI- 0100 佐劑對亞單位流感疫苗的作用)
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GPI-0100, a semi-synthetic saponin derivative, has been shown to be stable with a long shelf-life and possess adjuvant activity that could stimulate both humoral and cellular arms of an immune response. The incorporation of GPI-0100 into influenza virus vaccines provides a potential way to overcome several limitations we confronted with the current available vaccines and to help achieving the considerable demand for vaccine manufacturing for both influenza epidemics and pandemics. Our studies show that GPI-0100 could enhance antibody inducing capacity and cytokine (IFN-γ and IL-4) secretion capacity of whole inactivated virus (WIV), subunit (SU) and virosme (SV) based H5N1 (NIBRG) vaccines. Remarkable immune responses were especially identified with the adjuvanted subunit vaccines in a GPI0100 dose dependent manner. A further dose combination study with H1N1 (A/PR8) subunit vaccine showed that supplementation of GPI-0100 significantly enhanced the antibody titers and could be achieved comparably even at an extremely low antigen dose (0.04 ug). All humoral responses elicited were Th2 skewed responses. The presence of antigen itself in vaccines was important for viral load control but the antigen dose and the presence of GPI-0100 showed no particular influence on this aim. The dose combination of 0.04 ug HA+30 ug GPI-0100 represented the best vaccine formulation observed from the present study.

A novel combination vaccine for the control of seasonal influenza (一種控制季節性流感的新型聯合疫苗)
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Induction of influenza specific neutralising antibodies is the most effective type of immunity to combat influenza infection. Vaccination with inactivated detergent-split virus can induce Ab-mediated protection against disease but the efficacy is low, particularly in the elderly. In addition, protection can be compromised if the vaccine strains are a poor match with the circulating virus as a result of antigenic drift. Significant improvements could be made if the vaccine were to induce a cross-protective response to conserved elements of the virus. Another improvement would be to decrease the antigen dose required to give protection. To address these issues, we have tested a novel combination vaccine consisting of a sub-optimal dose of the current split virus vaccine and a cross-protective T-cell inducing lipopeptide. The lipopeptide contains Pam2Cys, which is a Toll-like receptor 2 ligand, and stimulates DC maturation and $CD4^+$ and $CD8^+$ T cell induction to attached peptide epitopes.
Mice immunised with this vaccine showed superior levels of lung viral clearance after challenge, compared to split virus alone. The addition of the lipopeptide to an even lower and

non-protective dose of split vaccine, also allowed pulmonary viral clearance. Lung titres were reduced by 10-fold for parenteral delivery of the vaccine and 1,000-fold for nasal delivery. This immunisation strategy not only provides protection at lower doses of split virus, but could also help in the event of a novel subtype of virus entering circulation by inducing some level of cross-protection against strains not present in the vaccine.

Influenza virus activates inflammasomes through intracellular M2 channel (流感病毒透過細胞內的 M2 通道激活過敏體(inflammasomes))

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Influenza virus, a negative stranded RNA virus causing severe illness in humans and animals, stimulates the inflammasome through the NOD-like receptor (NLR), NLRP3. However, the mechanism by which influenza virus activates the NLRP3 inflammasome is unknown. Here, we show that the influenza virus M2 protein, a proton-selective ion channel important in viral pathogenesis, stimulates the NLRP3 inflammasome pathway. M2 channel activity was required for influenza activation of inflammasomes, and sufficient to activate inflammasomes in primed macrophages and dendritic cells. M2-induced inflammasome activation required its localization to Golgi and pH gradient. Our results reveal a mechanism by which influenza virus infection activates inflammasomes, and identifies the sensing of disturbances in ionic concentrations in intracellular vesicles as a novel pathogen recognition pathway.

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肆.會議心得

這是一個世界性的免疫學術研討會,每三年舉辦一次; 今年為第十四屆,在日本神戶市舉行。今年出席這次大會約有來自 76 個國家和地區共 6000 人,大會為期 6 天; 包括 5 天全天候,超過 4000 篇學術研討會及壁報展示論文; 是國際免疫學大會一個最成功的紀錄。

個人專長領域並非免疫學,但因從事新興傳染病的研究,特別是各項傳染性致病原疫苗的研發與生產;自然需對免疫學有基本的認知,才能進行相關的研發與產品評估。 很高興能參與這個國際免疫學研討會,個人參與學術壁報展示,題目為「新型流感 H1N1 疫苗對小鼠之保護性免疫反應研究」(編號 2010-A-2803-ICI);光是和流感病毒相關的研究題目即超過 50 個 (如報告內之擇錄摘要),研究內容包含甚廣: 如

1.在越南兒童感染禽流感(H5N1)所引發急性呼吸窘迫症候群之關鍵細胞激素/趨化因子;
2.白細胞介素 15 是流感病毒引起的急性肺損傷發病機制的重要因子;
3.流感肺炎致病機制與中性粒細胞髓過氧化物酶(MPO)的功能有關;
4.老人接種人類流感疫苗產生之 B 細胞反應的內在缺陷;
5.鼻流感疫苗含有體 pFL 和 CpG 寡脫氧核苷酸作為黏膜佐劑引起的 S - IgA 抗體交叉反應功能;

6.季節流感及 2009 年 H1N1 疫苗免疫產生之抗體反應；

7.流感病毒感染期間傳統的樹狀細胞會提供缺乏漿樹狀細胞補償性黏膜免疫；

8.小鼠感染流感病毒肺臟中自然殺手細胞增生與成熟的標誌(KLRG1)之表現；

9.巨噬細胞腫瘤壞死因子相關凋亡誘導配體(TRAIL)的表達為干擾素 -β-依賴性可導流 10.感病毒性肺炎肺泡上皮細胞凋亡和屏障功能的障礙；

11.漿樹突狀細胞的免疫原性劃定流感疫苗亞型；

12.流感病毒感染引起的免疫反應與患者的發病機制相關；

13.感染豬，禽和人類 2009 年的流感病毒，與聚- IC 的刺激分別上調體外豬的樹突狀細胞表面標誌和細胞激素的分泌；

14.分析長 pentraxin 3 作為豬生物標誌物；

15.人類單核細胞樹突狀細胞對合併感染流感病毒和肺炎鏈球菌的反應；

16.I 型干擾素的信號是流感病毒感染後控制肺單核細胞和中性粒細胞所必需的；

17.從非致病性病毒庫來之滅活性完整顆粒疫苗賦予食蟹獼猴 H1N1 流感病毒保護性免疫預防比不分裂疫苗更有效；

18.感染流感病毒後繼發肺炎球菌性肺炎，表面蛋白 A（PspA）對肺炎球菌的免疫保護作用；

19.酵母菌瘁取物 Zymosan 透過提高粘膜佐劑活性增加抗流感病毒

分泌性 IgA 抗體生產；需要 Toll 樣受體（TLR）信號活化 T 細胞獨立性病毒特異性記憶 B 細胞；

20.H1N1 流感大流行的免疫反應縱世代研究;宿主對抗流感(H5N1 和 H1N1 病毒）感染；

21.virosome 為基礎的禽流感疫苗具有潛在的免疫原性；

22.雞干擾素λ作為抗病毒治療；

23.人單核細胞衍生之樹突狀細胞對合併感染流感病毒和肺炎鏈球菌的反應；

24.I 型干擾素的信號是流感病毒感染後控制在肺單核細胞和中性粒細胞所必需的；

25.流感引起肺組織損傷需要 IL - 1 信號進行修復；

26.人類流感病毒較禽流感病毒能有效引起支氣管上皮細胞的抗病毒抑制作用；

27.使用脂溶性染料標記的流感病毒研究肺部樹突狀細胞功能；

28.豬源流感病毒（S-OIV）疫苗對小鼠保護性免疫反應研究；

29.組合四聚體染色和質譜儀對人類和小鼠的流感特異性細胞毒性 T 細胞反應進行研究；

30.甲型 H1N1 流感大流行引起的住進加護病房重症呼吸疾病個體 CCR5δ32 等位基因頻率增加；

31.疫苗志願接種者周邊血獲得的人抗流感 M2 抗體特性研究；

32.產生對病毒基質蛋白 1(M1)的 transbody 以作為流感的治療；

33.舌下接種流感活病毒誘導持久保護性免疫；

34.記憶潛力是由抗原表位的數目決定；

35.在感染流感後記憶 CD8＋T 細胞的發展和分化；

36.布魯頓的酪氨酸激酶(Bruton's tyrosine kinase)參與鼻腔接種流感疫苗的免疫反應；

37.利用植物為基礎的流感及艾滋病疫苗防治策略優點：較好的免疫原性，更耐熱，穩定，快速的瞬態蛋白生產；

38.研製 T -細胞誘導的泛流感疫苗；

39.大流行流感（H1N1 病毒）病毒樣顆粒（VLP）疫苗對人類是安全的且具高度免疫原性：第 2 期臨床試驗研究；

40.以特異性 IgY 治療可控制季節性和大流行性流感病毒且不會干擾適應性免疫的發展；

41.植物熱休克蛋白 70 作為植物表達的重組抗原的傳遞載體；

42.Mekabu 褐藻多醣硫酸酯的免疫刺激作用:依中老年人可產生抗流感疫苗抗體作評估；

43.在小鼠評估並優化 GPI- 0100 佐劑對亞單位流感疫苗的作用；

44.一種控制季節性流感的新型聯合疫苗；

45.重組鞭毛蛋白優越的療效：決定病毒 HA 球狀頭部疫苗安置在鞭毛球形頭部的效果；

46.脂質體包含疫苗與 β-葡聚醣的混合物鼻腔給藥之效果；

47.H5N1 禽流感的免疫刺激性 RNAi 治療；

48. 發展誘導 CTL 對抗甲型流感病毒的肽疫苗；

49.流感病毒透過細胞內的 M2 通道激活過敏體(inflammasomes)

等。

這些研究對個人在流感病毒的研究、疫苗的研發與製造、疫苗品管與確效的評估、免疫佐劑的使用及效果評估、動物試驗的設計及其免疫反應評估、疫苗申請進入臨床試驗程序等等均有很大的啟發與助益。總之，參與這個國際免疫學研討會對個人有很大的助益；希望日後仍有機會可參與。

伍、回單位後報告情形

回單位後利用時間在實驗室與研究助理討論，將擇期於大組會議進行報告；同時設計新的實驗來評估疫苗的交叉保護作用。

陸、建議事項

一、國際免疫學大會(International Conference of Immunology ,以下簡稱 ICI) 是一個世界性的免疫學術研討會，每三年舉辦一次。預防醫學研究所從事新興傳染病的研究，特別是各項傳染性致病原疫苗的研發與生產；自然需對免疫學有基本的認知，才能進行相關的研發與產品評估。無論在免疫學或流感病毒的研究、疫苗的研發與製造、疫苗品管與確效的評估、免疫佐劑的使用及效果評估、動物試驗的設計及其免疫反應評估、疫苗申請進入臨床試驗程序等等均有很大的進展與創意，對於參予者有很大啟發與助益。總之，參與這個國際免疫學研討會對個人有很大的助益；希望日後同仁仍有機會可

參與。

二、有鑑於學術研究的交流能啟發所有教師及研究人員的研究創意，國內外各學校及研究機構莫不鼓勵其同仁盡量參加，包括各項獎勵補助及預算編列。學院自從縮減參加國際研討會的預算補助後，已經嚴重影響同仁參加的意願；個人以為一個教學醫學院須有宏觀的前瞻性，如再不加強鼓勵同仁參加，必定會使學院的研發能量下降。 因此，個人建議軍醫局應鼓勵增加補助同仁(包括研究助理)盡量參加各項國際研討會；包括各項獎 勵補助及預算編列。

三、由文獻及此次研討會,得知目前國際上在流感疫苗研發上主要有幾個方向:

1. 疫苗株改造之開發研究與利用: 主要利用 reverse genetic 技術建構較佳(病毒產量高、免疫抗原性強且具廣效性、副作用小)之重組病毒; 但病毒產量與副作用,免疫專一性與廣效性;往往一體兩面,很難雙全! 目前來自英國 NIBSC 的禽流感疫苗株(NIBRG-14)即有產量不夠高及免疫抗原性強問題。目前的作法是開發免疫佐劑的研究。
2. 開發具有趨向細胞性免疫(Th1)的免疫佐劑: 目前唯一經美國 FDA 同意用於人類的免疫佐劑為 alumn (aluminum hydroxide, aluminum phosphate), 但因其主要促使的免疫作用以抗体免疫(Th2)為主,因而可能產生很強之 IL-4 等免疫作用,連帶使身體產生各種不適等副作用! 開發具有趨向細胞性免疫(Th1)的免疫佐

劑除可降低疫苗劑量外,更能有效的消除病毒;且其免疫反應所產生之副作用也較小。本次研討會即有相關的研究，如流感病毒相關的研究題目項次 5、13、19、36、37、41、42、及 43 等，均在探討相關之研究。

3. 以基礎研究(結合 reverse genetic 技術及疫苗病毒株改造)找出影響病毒產量、病毒毒性、跨物種感染的病毒基因及宿主(禽類或人類)因子, 這是流感防治的根本解決之道。 目前個人實驗室即找到細胞中特定蛋白可以和流感病毒核酸聚合酶 PB2 結合，透過此蛋白影響病毒之轉錄及複製：且不同流感病毒之核酸聚合酶 PB2 結合至此蛋白的差異與其病毒聚合酶活性及其在細胞中之複製能力呈正相關。
4. 疫苗生產方式的研究: 主要理由是當禽流感病毒流行時,家禽可能大量死亡,因此無足夠的蛋可供疫苗製備。其次是長久以來存在的蛋白所引起之過敏性反應問題! 再其次是以蛋生產疫苗,其病毒培養、純化、及自動化產程等均十分不便。因此有許多疫苗製造公司利用美國 FDA 同意的細胞株(MDCK, Vero),並結合微粒(microcarriers)培養方式,或製成 virosome 等方式;進行疫苗之生產與製造。如流感病毒相關的研究題目項次 21、39 等；目前這是新的禽流感疫苗生產方式趨勢。
5. 免疫方式與免疫途徑等研究: 如鼻腔噴劑疫苗之開發(如流感病毒相關的研究題目項次 5、46 等)、舌下接種疫苗、病毒樣顆粒(VLP)疫苗、以植物(番茄)生產之蛋白疫苗、重組鞭毛蛋白、仿

蚊子叮咬針頭之開發、脂質體包含疫苗與 β-葡聚醣的混合物、貼布疫苗之開發,..等。

6. 治療性產品，如流感病毒相關的研究題目項次 22、32、40 等。

7. 其它:

柒、參加此會議對單位之貢獻

參加此研討會除可增加個人的專業知識外,還可經由瞭解目前國內外相關研究的方向及最新進展;對本所禽流感相關研究及疫苗製造方式及產線規化有很大之助益。 個人由參加此研討會,認為本所禽流感疫苗研發重點之優先順序為:

1.建立各種合作管道(與中研院、國衛院、疾管局、淡水家畜試驗所等合作),取得人類及禽流感病毒及疫苗株;

2.建立 reverse genetic 技術平台並結合疫苗病毒株改造研究(約需 3~4 人); 優先評估禽流感病毒基因 NP, NS1,及 PB2 等置換後對病毒之產量、病毒毒性、跨物種感染的影響; 目前個人實驗室已建立此能力；可以由疫苗株 HA 抗原進行基因改造重組出較毒或致病力較高的病毒；也可反向由野生病毒進行改造重組出疫苗株等。

3.建立禽流感病毒免疫及其效果評估的研究團隊(約需 4~5 人); 從免疫方式與免疫途徑等研究、病毒生產及純化、病毒效價及疫苗劑量之定性及定量、動物之免疫及攻毒等工作, 均需一組有經驗的團隊來執行;

4.抗病毒藥物篩選或抗病毒分子(siRNA, peptide, 人類化治療用單株抗體,分子複合物等)研發;

5.疫苗生產方式的改良研究, 著重如何在特定疫苗株狀況下提高病毒生產量;

6.免疫佐劑之開發及評估;

7.加強疫苗之品管及品保，取得各項測試方法之 GLP 認證；致力使疫苗通過 GMP 認證。

8.加強不同致病原同時感染引起之免疫機轉及副作用研究；

9.其它。

總之,個人認為參加此研討會對本所相關任務及工作有很大之助益;相信藉由研討會之新知可協助瞭解最新之知識及方向,進而對研究及生產產程產生更好的想法與作法。 對研究助理而言也可進行知識及技術之培訓,滿足求知慾;進而增進工作熱忱與效率。

捌、附件資料