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寒熱藥對氣喘鼠模型體質之基因體差異  
Genomic expression of asthma animal model in heat-TCM  
or cold-TCM

研究報告

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## 附件 2

# 寒熱藥對氣喘鼠模型體質之基因體差異

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### 摘要

研究目的:基於寒證用熱藥，熱證用寒藥之基本識認，看治療氣喘之寒藥及熱藥對氣喘鼠的 gene 之不同的表現，來探討其寒熱不同的分子層面

研究方法:將老鼠分經 PBS 或塵蟎致敏小鼠後，連續餵食熱藥小青龍湯或麻杏甘石湯(隔天餵食 1g/kg，六次)，之後分別以 PBS 或塵蟎激發，用肺泡沖洗液中的細胞種類之變化做為指標，取出肺組織，轉成 cDNA，再用 macroarray 區別，為氣喘鼠在誘發後之 gene 現表上的變化。綜合上述實驗結果，希望能從肺組織之 gene 表現的差異，看寒藥或熱藥對氣喘鼠之模型對其 gene 的變化來做比較，在寒藥或熱藥影響下 gene 的表現的改變，以此推出寒證氣喘與熱證氣喘之 gene 表現之差異，以期能更進一步對塵蟎致敏之患者利用中醫藥於緩解、調整與治療氣喘的療程中，gene 表現因寒熱的狀態不同來證明寒熱對體質上的影響。

結果與討論:經基因體研究，發現經塵蟎所誘發的氣喘鼠有 568 個基因被誘發升高，有 84 個基因被降低表現；而經小青龍湯治療後，有 121 個基因被調降，而有 51 個基因被調升；另外，經麻杏甘石湯治療後，11 個基因被調降，而有 31 個基因被調升。由其中選出明顯相關的基因，進行 RT-PCR 的驗證，首先呈現的是 TLR-2 被 Der-p 刺激而表現持平，卻被小青龍湯明顯誘發，而麻杏甘石湯誘發比較差，由此可臆測中藥壓制了 mite 過敏源刺激 APC 產生 Th2 路徑，而減少 IL-4、IL-5、IL-13 之表現，進而減少 IgE 的表現，而降低由 mite 誘發的氣喘反應。而 CD14 上升，被小青龍湯壓制，但麻杏石甘湯只有部分壓制，反應有所不同，小青龍湯對 NF-kB 及 C-fos 亦有明顯抑制，其餘相關基因將陸續驗證。

關鍵詞：氣喘動物模式，小青龍湯，麻杏石甘湯，CD14，TLR-2，NF- $\kappa$ B

附件 3

## Genomic expression of asthma animal model in heat-TCM or cold-TCM

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### ABSTRACT

**Aim:** In an effort to provide unbiased insight into Tradition Chinese Medicine (TCM) treatment, we design an approach involving transcript expression profiling of lung tissue from mice with experimental asthma. We were interested in reproducibly and accurately identifying genes that were differentially expressed in a well-established model of asthma.

**Method:** We developed chronic allergic mice in intratracheal (i.t.) instillation House dust mite Der-p for a month. The model was challenged with Der-p every weeks for a months. In TCM therapy group, we give 1g/kg of TCM before Der-p-challenged. We analyzed the expression of genes identified in the mouse studies in mouse lung from subjects with none, Der-p induced asthma, Der-p induced asthma treated with heat-TCM XQLT, and Der-p induced asthma treated with cold-TCM MXGST. Whole-lung RNAs were purified by using RNeasy® Midi Kit (Qiagen). Integrity of all RNA samples used in this study was confirmed with a model 2100 bioanalyzer (Agilent Technologies, Inc, Palo Alto, Calif). Cy3-labeled and Cy5-labeled lung cDNAs were prepared as described(5). Using a microarray, we examined changes in gene expression in asthmic lung, with heat-TCM XQLT, and Der-p induced asthma treated with cold-TCM MXGST following exposure to an organic extract of Der-p.

**Results & Discussion:** We used DNA microarrays to analyze gene expression in mice models of asthma and TCM-treated. In whole-lung samples, 568 gene transcripts were differentially expressed (increased or decreased) between Der-p induced chronic asthma mice and naïve mice. Of these, 84 genes were increased or decreased by 1.5-fold and 0.5-fold ( $Cy3 > 100$  or  $Cy5 > 100$ ,  $Prob > 0.95$ ) in asthma model. We compare the Der-p induced data to XQLT-treated data in 172 genes. We demonstrate the pattern of gene differentially expressed between Der-p induced or suppressed to TCM-treated. 121 gene was suppressed by XQLT and 51 genes was up-regulated by XQLT. the Der-p induced data to MXGST-treated data in 47 genes. We demonstration the pattern of gene differentially expressed between Der-p induced or suppressed to TCM-treated. 11 gene was suppressed by MXGST and 36 genes was up-regulated by MXGST. Mite allergen stimulation of APCs normally tends to promote a Th2

lymphocyte response by secretion of interleukin-10, IL-4, IL-5, and IL-13. CD14 and TLR-2 on the cell surface of APCs was induced by XQLT and MXGXT to regulated the Th2 response.

Keywords : Mite, Ma-Xing-Gan-Shi-Tang,Xiao-Qing-Long-Tang,gene expression,CD14, TLR-2

## 壹、前言

氣喘是全球性的重大公共衛生問題，其罹患率與致死率在過去二十年持續增加，其中以在西方國家最為明顯[1-2]，雖然增加的原因並不清楚，但過敏與氣喘之間有很清楚的關係存在[3]。台灣處於亞熱帶地區，因氣候潮濕，罹患過敏性疾病甚多，其中以過敏性鼻炎與氣喘最為常見。近年來，環境污染日益嚴重，過敏性疾病如支氣管氣喘、過敏性鼻炎、濕疹，呈現增加的趨勢。根據調查，近 20 年間(民國 63 年至 83 年)，台北地區學童氣喘流行率增加約八倍[4-5]，由此可見對氣喘治療與預防的重要性。根據研究報告指出，氣喘會造成支氣管平滑肌收縮、發炎細胞、發炎介質的參與及神經性調節之交互作用，並引發呼吸道發炎與支氣管的持續過度反應和不同程度的呼吸道阻塞[6-9]。近年來許多研究傳統中醫藥經由調節免疫系統(例如 T 輔助型細胞[Th]中 Th1/Th2 細胞之調控等)，而達到“改善體質”之作用來達到預防與治療氣喘的目的，我們研究發現，中藥方劑可以降低氣喘天竺鼠呼吸道過度反應性、立即性與遲發性呼吸道阻力，調節發炎細胞浸潤與氣喘老鼠肺周邊淋巴結(drainage lymph node ; DLN)中 Th2 細胞之百分比[10-15]。

氣喘致病機轉中，T-淋巴球在產生、調控及氣喘呼吸道慢性發炎上扮演重要角色，其暴露於過敏原後，肥大細胞(mast cell)開始了發炎之過程，淋巴球很可能提供氣喘活化進而建立慢性且持續性發炎之訊息。我們的研究發現小青龍湯可調控 T 淋巴球之浸潤[13]，而動物模式亦顯示由塵蟎(*Dermatophagoides pteronyssinus*, Der p)所激發之氣喘模型中，Th2 細胞(IL-4<sup>+</sup>/CD3<sup>+</sup>/CD4<sup>+</sup>)之百分比亦明顯增加[11]。由前人之研究中可看出輔助性 T 輔助型淋巴球依所分泌淋巴激素不同可分成 Th1 及 Th2，Th1 細胞產生 IL-2、IL-3、GM-CSF、INF- $\gamma$ 、IL-10、TNF- $\beta$  等細胞激素，Th2 細胞產生 IL-3、GM-CSF、IL-4、IL-5、IL-6、IL-10、IL-13 細胞激素，這二種次分類的 Th 細胞參與不同的免疫反應，Th1 細胞參與遲發性過敏反應 (delayed type hypersensitivity)而 Th2 細胞參與過敏性發炎反應(allergic inflammation)。

此外，在 cytokine、chemokine 與發炎細胞之間的相互影響中，人類 Th2 細胞除了選擇性製造 IL-4、IL-5、IL-6、IL-9、IL-10、IL-13 外，並表現優先釋放 macrophage-derived chemokine (MDC)[26]，MDC 可能會促進過敏性發炎[27]，進而使 Th2 細胞表現 CD30、CCR4、CCR8、及 CRTH2，這些 chemokine receptor 和 Th2 細胞趨化並聚集於標的組織(recruitment & homing)有關[28]，最近幾年，影響特異性免疫反應導致 Th1 或 Th2 路徑的因子已廣泛被發現，

Th1 及 Th2 細胞是由相同之 T 輔助性細胞前驅者於抗原呈現時在環境與遺傳因素雙重影響下發展出來的。Th1/Th2 分化主要是調節一群 contact-dependent factors (CDF) 與特定細胞激素在佔支配地位。CDF 中最重要的為(1)TCR ligation [29]，(2) OX40L-OX40 及 B7-CD28 相互反應所釋放的信息[30-31]，OX40 共同刺激增強 IL-4 在引導及促進原始 CD4 T 細胞分化成高 IL-4 製造作用器之表達[30]。雖然 B7-CD28 相互作用複雜且尚無定論，但是原始 T 細胞製造 IL-4 似乎高度依賴 B7 分子[31]若 IL-4 誘生效應超越其他細胞激素，一旦 IL-4 濃度達到必需閾值，T 輔助性細胞分化成 Th2 之基因表型於是產生。另一方面，若早期由樹突細胞 (dendritic cell) 產生的 IL-12、IL-18、及 IFN ( $\alpha$ 、 $\gamma$ ) 於反應性 Th 細胞微環境中大量表現，則 Th0 會發展成 Th1 細胞，尤其是 IL-12 [32-35]。

中醫認為氣喘急性發作期，治療應以調節肺、脾二臟為，應以治標也就是平喘為優先。分為寒、熱二大證型，寒證可用小青龍湯，熱證可用麻杏石甘湯。小青龍湯及麻杏石甘湯是東漢張仲景《傷寒論》治療寒證氣喘的名方，我們研究發現熱藥小青龍湯具有降低卵白蛋白(OVA)誘發氣喘天竺鼠立即性與遲發性氣喘反應，減少 OVA 誘發氣喘老鼠肺泡沖洗液發炎細胞及嗜酸性白血球浸潤百分比，OVA 激發前 30 分鐘餵食熱藥小青龍湯、激發前 30 分鐘與激發後 5 小時均餵食熱藥小青龍湯及致敏開始即每天餵食熱藥小青龍湯能顯著降低立即性與遲發性氣喘反應的呼吸道阻力，其中激發前 30 分鐘餵食熱藥小青龍湯天竺鼠於激發後 2 至 8 小時的各時間點，激發前 30 分鐘與激發後 5 小時均餵食熱藥小青龍湯的天竺鼠於激發後 4 至 72 小時的各時間點能降低呼吸道阻力，致敏開始後即每天餵食熱藥小青龍湯的天竺鼠則自激發 1 小時後的各時間點都與正常天竺鼠無顯著差異。卵白蛋白激發前 30 分鐘給熱藥小青龍湯、激發前 30 分鐘與激發後 5 小時均給熱藥小青龍湯的天竺鼠於激發後 6 小時與 24 小時其嗜酸性白血球的比率顯著低於未經治療的氣喘天竺鼠，肺部病理切片則顯示，經熱藥小青龍湯處理之天竺鼠能降低發炎細胞的浸潤並降低平滑肌收縮的程度[12]；將熱藥小青龍湯經口服餵食無致敏小白鼠(1g/kg-mouse) 24 小時後，其淋巴球於肺沖洗液中明顯比無餵食熱藥小青龍湯組多出約 30 倍之淋巴球總數，相對百分比增加了 10 倍，進一步之表面抗原分析結果顯示肺泡沖洗液中 CD3<sup>+</sup>/CD4<sup>+</sup>T 細胞百分比降低而 CD3<sup>+</sup>/CD8<sup>+</sup>與 CD3<sup>+</sup>/CD4<sup>-</sup>/CD8<sup>+</sup>T 細胞百分比明顯升高，已明顯指出熱藥小青龍湯在調節 T 淋巴球浸潤過程中扮演重要角色，熱藥小青龍湯口服餵食無致敏小白鼠亦增加巨噬細胞與嗜中性白血球數目，但巨噬細胞之相對比例卻明顯下降，其原因為相對於淋巴球之數目大增之關係，熱藥小青龍湯影響淋巴球、巨噬細胞與嗜中性白血球於無致敏小白鼠之肺部浸潤；重點是無論是在臨床上之觀察與實驗室之研究均證實熱藥小青龍湯確實可改善氣喘症狀，如塵蟎致敏的天竺鼠與小白鼠之動物模型中都確切指出其



抑制嗜酸性白血球浸潤，證明熱藥小青龍湯對已具有過敏體質之個體具有明顯的治療功效，其中於塵蟎致敏之小鼠經連續給予熱藥小青龍湯兩週後以塵蟎激發後，其肺泡沖洗液中總浸潤細胞數、巨噬細胞、淋巴球、嗜中性白血球與嗜酸性白血球數目均明顯降低，但淋巴球之相對百分比明顯升高[13]；而寒藥麻杏石甘湯能有效治療熱性氣喘，其可緩解氣喘天竺鼠立即性反應期之呼吸道阻力，降低呼吸道炎症。對遲發型呼吸道反應期的嗜酸性白細胞沒有明顯的抑制作用，在塵蟎致敏的天竺鼠立即型的氣喘反應中(immediate asthmatic responses, IAR)，在第 1, 6 及 24 小時有明顯的 neutrophil 增加。兩者在氣喘鼠的身上似乎有著不同的表現，其不同之表現在基因上的差異又是如何？這樣的差異是否決定了對氣喘之療效或是不同的體質產生不同的反應；由以上的疑問促使我們設計這個實驗，希望進一步了解熱藥小青龍湯或寒藥麻杏石甘對正常老鼠及塵蟎誘發氣喘老鼠肺部之 cytokine、chemokine、adhesion molecular、transcription factor、signal transduction associated protein and oncogene 廣泛的調節作用，進而影響到免疫組織間的微環境做為比較，基於寒證用熱藥，熱證用寒藥之基本識認，看寒藥及熱藥對氣喘鼠的 gene 之不同的表現，來探討其分子機轉。將老鼠分經 PBS 或塵蟎致敏小鼠後，用肺泡沖洗液中的細胞種類之變化做為指標，抽取 total RNA，轉成 cDNA，再用 macroarray 區別，為氣喘鼠在誘發後之 gene 現表上的變化。

希望用 macroarray 能從肺組織之 gene 表現的差異，看寒藥或熱藥對氣喘鼠之模型對其 gene 的變化來做比較，在寒藥或熱藥影響下 gene 的表現的改變，以此推出寒證氣喘與熱證氣喘之 gene 表現之差異，以期能更進一步對塵蟎致敏之患者利用中醫藥於緩解、調整與治療氣喘的療程中，gene 表現因寒熱的狀態不同來證明寒熱對體質上的影響。

## 貳、材料與方法

### 一、藥品製備與購買

#### I、中藥製備

寒藥麻杏石甘湯(Ma-Xing-Gan-Shi-Tang, MXGST)及熱藥小青龍湯(Xiao-Qing-Long-Tang, XQLT, Sho-Seiryoto), KODA pharmaceutical Co., Ltd. in Taiwan) 是由 8 種中藥材組成如 Table 1, 600 公克中藥材加水 6 公升，室溫浸泡 30 分鐘後，煮沸 50 分鐘。原藥材重複煎 2 次，並將 2 次藥水混合，以 No.1 濾紙過濾，收集濾液，濃縮到 600 毫升，取 1ml 秤重後冷凍乾燥，計算乾重。之後將淨膏。稀釋成 100mg/ml，儲純於-30°C 冰箱中備用。

## II、藥品之購買與製備

將 1 克的家塵蟎粉末(*Dermatophagoides pteronyssinus* [Der p])購自 Allergon (Engelholm, Sweden) )倒進 200ml 的錐形瓶內，加入 100ml 的乙醚，瓶口蓋上錫箔紙，放置於通風櫥中以電磁棒攪拌一天或者直至乙醚完全蒸發為止，再加入 100ml PBS。將家塵蟎粉末溶液倒進鉢中研磨，不定期取少量溶液置於玻片，在顯微鏡下觀察，一直研磨到家塵蟎呈大小均一的碎片，即停止研磨。之後，在 4°C 下將家塵蟎溶液攪拌 2 天，再倒至 50-ml 的離心管，以 12000rpm 轉速，在 20°C 下，離心 30min，以吸管將淡棕色上清液轉移到 50-ml 離心管，管口蓋上事先處理過的透析膜(dialysis membrane, cut off point: 12-14 KDa)，在 4°C 中以 2 升的 RO 水透析 2 天，每隔 8 小時換水一次。透析以後的透析液在 4°C 下以 3000 rpm 轉速離心 10 分鐘，取上清液，每 10ml 分裝至事先稱好的 50-ml 離心管，然後置於 -70°C 冰箱，隔天取出以冷凍乾燥機乾燥直至水份蒸乾。完成後再稱重(包括離心管)，以冷凍乾燥後的重量扣掉空試管的重量，即是冷凍乾燥家塵蟎的重量。以無菌生理食鹽水回溶，並調節為 1.6mg/ml 及 1.0mg/ml 兩種濃度，之後再以 0.22  $\mu$ m 的濾器過濾後，每 1ml 分裝一瓶，儲存於 -20°C 冰箱備用。在每次製備完成之家塵蟎溶液皆使用 *Limulus ameobocyte lysate test* 貞測 LPS 的含量，在每次製備完成之塵蟎溶液之 LPS 含量皆低於 0.96Eu/mg Der f。

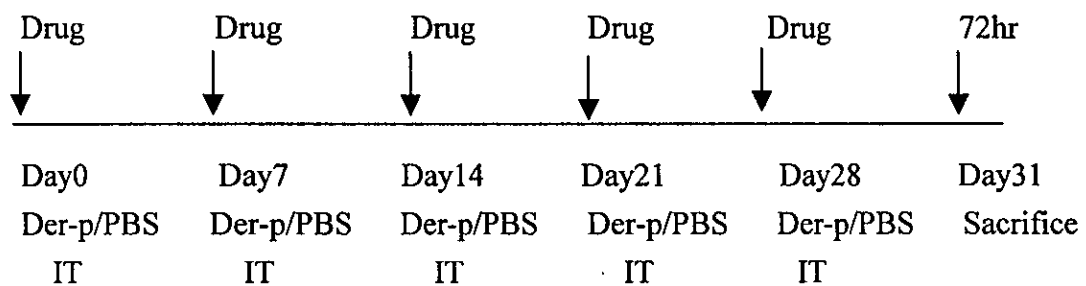
## 二、實驗分組

將老鼠分為下表之四組(Table 1, A-D 組)，經 PBS 或塵蟎致敏小鼠後連續餵食小青龍湯或麻杏石甘湯(隔天餵食 1g/kg，六次)，之後分別以 PBS 或塵蟎激發(Fig. 1)，我們將探討肺部組織中各種 cytokine and chemokine gene(IL-1、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  及 IL-2, IL-4、IL-5、IL-6、IL-9、IL-10、IL-13), inflammatory signal transduction gene (NF- $\kappa$ B pathway, MAPK pathway), transcription factor(STAT gene, cMaf gene and GATA gene), cell adhesion molecular (ICAM-1 VACM-1 and E-selectin), and cell cycle-regulatory gene (p21, p53, CD2,.....).之 gene 變化。

Table 1

組別		塵蟎致敏 (50µg/mouse)	餵食小青龍湯或麻杏石甘湯 (1g/kg-mouse/time)	塵蟎激發 (50µg/mouse)
A	Naïve	-	-	-
B	Derp	+	-	+
C	Derp+小青龍湯	+	小青龍湯	+
D	Derp+麻杏石甘湯		麻杏石甘湯	+

Fig. 1 Der p challenged chronic asthmatic protocol



## 二、RNA 抽取及 cDNA 合成

取出小鼠肺組織後以 TRIzol(Invitrogen, Carlsbad, CA) 抽取 RNA，方法如下：

1. 肺組織經冰凍研磨後加 1.5 ml TRIzol Reagent.
2. Pipette 反覆抽吸數次(打破細胞)，靜置 5min，刮下，轉放到 eppendrof。
3. 加 0.3 ml 氯仿 (or chloroform-isoamyl alcohol) 震動攪拌 15 秒。
4. 離心 12,000 xg, 10 分鐘, 4°C。
5. 移上清液到 RNA 用 eppendroff, 約略測上清液體積。(其餘丟掉)

6. 加體積比 1:1 Isopropyl alcohol。翻轉混合 samples，靜置 10min 在 4°C。
7. 離心 12,000 x g, 4°C. RNA pellet 會出現底部。
8. 小心倒掉上液。再加 Add 1ml 95% ethanol 及 0.45 ml TRIzol Reagent 清洗 RNA pellet. (可以 vortex)。
9. 再離心 12,000 x g，10 分鐘，2-8°C。
10. 小心倒掉上液，陰乾 RNA pellet。(不可過乾)
11. 加 DEPC H<sub>2</sub>O 放置於 -80°C 溶解備用。

### 三、MicroArray

#### *RNA 收集和 array hybridization 探針製備*

利用 TRIzol reagent (Invitrogen Life Technologies, P/N 15596-018) 抽取小鼠全肺組織 並且以 Rneasy Mini Kit (Qiagen, P/N 74104) 清洗乾淨，再利用 T7-Oligo(dT)<sub>24</sub> primer (5-GGCCAGTGAATTGT AATACGACTCACTATAGGGAGGCGG-(dT)<sub>24</sub>-3) 合成 cDNA，雙股 cDNA 進一步以 Phase Lock Gel (Eppendorf, P/N 0032 007.953)-phenol/chloroform extraction (Ambion, P/N 9732) 純化。接著在利用 Enzo RNA Transcript Labeling Kit (Affymetrix, P/N 900182) 標記 cDNA，被 biotin-labeled cDNA 利用 Qiagen Rneasy Mini Kit 純化，同時 cDNA 片段大小平均約 50-200 bases，並置於弱鹼中 94 °C、35 min。雜交溶液由 0.05 µg/mL cDNA 片段, 1 µg/mL herring sperm DNA, 1 µg/mL acetylated BSA 和 50 µg/mL hybridization buffer 組成。另外，雜交溶液中含有 4 種細菌和病毒的 control cDNAs (bioB, bioC, bioD and cre at 5, 5, 25 and 100 pmol/L) 當作 array 雜交效率的比較工具。在晶片角落和中心點特別利用 biotinylated oligonucleotide B2 雜交，來定位晶片的的方向和確認探真的位置。

#### *Array hybridization and scanning*

雜交混合物被加熱到 99 °C、5 分鐘，之後 45 °C、5 分鐘，離心 5 分鐘，將不溶物移除。晶片以適量的 1× hybridization buffer 浸濕後，在 1× hybridization buffer 中 45 °C 反應 10 min，之後將 buffer solution 倒掉，並加入雜交混合物。

cDNA 片段(50  $\mu$ g) 與 Affymetrix MOE430A array (containing 22 690 transcripts, almost 14 500 known genes and 4 371 ESTs) 在 Affymetrix®Fluidics Station 400 雜交 16 小時。晶片雜交結果以 GeneArray Scanner (G2500A geneArray Scanner, Affymetrix) 來掃描，cDNA 探針先以 MOE430A array 來作測試，並且先肯定其雜交的品質。

共進行下列組：

- I. NAÏVE 組.v.s.Derp 誘發組, 三片 genechip
- II. Derp 誘發組 v.s.寒藥麻杏石甘湯組, 三片 genechip
- III. Derp 誘發組 v.s.熱藥小青龍湯組, 三片 genechip

#### 四、製備 cDNA 及 reverse transcriptase (RT)-PCR 放大

小鼠全肺組織的RNA利用BRL reverse transcription kit and oligo (dT) 12–18 primer，反轉錄為cDNA，amplification solution 包含 50 mM KCl, 10 mM Tris-HCl (pH 8.3),和 2.5 mM MgCl<sub>2</sub>。專一性的 oligonucleotide primers (200 ng/sample) 和5  $\mu$ g/ml cDNA被加到緩衝溶液中。cDNA放大32cycle，在94°C 4分鐘Denaturation，55°C 45秒Annealing，72°C 45 sec elongation。取2g的 agarose，倒入錐形瓶，保鮮膜封口，刺數個洞，放進微波爐，加熱1.5min，倒入電泳槽中凝固，緩緩加入0.5 $\times$  TBE緩衝溶液。取10  $\mu$ l DNA Sample加入2  $\mu$ l 6 $\times$ gel loading buffer，加入電泳膠片凹槽中，100volts 1小時，取出電泳膠片放入Ethidium bromide 30min，再放入水中進行脫色10min，用UV光激發並拍照。

#### 五、統計分析

所有結果以平均值 $\pm$ 標準差來表示，利用 SPSS 軟體來作統計分析，並採用 one-way ANOVA，比較各組間之差異。P 值小於 0.05 視為具統計上的差異。

## 參、結果

已成功建立以塵蟎(Der p)誘發之慢性氣喘 BALB/c 小鼠模型，麻杏石甘湯或小青龍湯確實可抑制慢性氣喘小鼠肺泡沖洗液中 eosinophil 之浸潤，肺組織病理切片亦發現麻杏石甘湯及小青龍湯可減少肺部發炎細胞浸潤及氣管壁增厚現象，(Table 2.Fig 2)。將各組肺組織萃取 mRNA 送國衛院分子與基因醫學研究室作基因體比較分析。

### Hematoxylin-eosin stain

Der p 組肺部發炎細胞浸潤及氣管壁厚度(Figure 2-B)，比 naïve 組(Figure 2-A)明顯增加。XQLT(Figure 2-C)和 MXSGT 組(Figure 2-D)比 Der p 組，有減輕現象。

### 基因晶片呈現的結果

#### 一、 Der-p 致敏組與 NAÏVE 組之比較

我們使用 DNA 晶片來分析中藥治療後氣喘小鼠基因表現情形，naïve 組和 Der-p 組比較，其中有 568 個基因被 up-regulated，84 個基因被 down-regulated (Fig A,B,C,D)。選取基因的標準是基因表現大於 1.5 倍 或基因表現小於 0.5 倍(Cy3 >100 or Cy5 >100, P < 0.05)。

#### 二、以 Der-p 致敏組與 Der-p + XQLT 組之比較

我們以 naïve 組和 Der-p 組比較中有 652 個基因有顯著差異為基礎，來比較 Derp 組和 XLQT 組，發現有 172 個基因表現有顯著差異，其中有 121 個基因被 up-regulated，51 個基因被 down-regulated (Table 3)。這些基因涉及各種不同的訊息傳遞路徑，例如 T-cell activation, proliferation, cytoskeletal changes, I $\kappa$ B $\alpha$ , TNF $\alpha$ , KC, MCP-3, IL-6, GM-CSF, defensin-1 and ribosomal protein L37, NGF 等等。

#### 三、以 Der-p 致敏組與 Der-p+ MXGST 組之比較

我們仍以 naïve 組和 Der-p 組比較中有 652 個基因有顯著差異為基礎，來比較 Derp 組和 MXGST 組，發現有 47 個基因表現有顯著差異，其中有 11 個基因被 up-regulated，36 個基因被 down-regulated (Table 4)。

#### 四、RT-PCR 再確定部份:

目前先進行比較明確的數個 gene : TLR-4, CD14, TLR-2 pathway(含 TAK, NF-kB, c-FOS, c-jun) 及各種 cytokine(TNF-alpha, IL-1beta, IL-10, IL-13, TGF-beta)及相關之 chemokines(Eotaxin, RANTES), 其 RT-PCR 結果如 Figure 3.

目前可由此結果推測出 Der-p 會誘發 TLR-2 及 CD14 表現, 而小青龍湯有明顯抑制此 receptor 之表現, 對其 pathway 有影響, 如麻杏甘石湯對 TAK 之表現有抑制的效果而小青龍湯對此基因表現不明顯, 但在 NF-Kb 及 c-fos 小青龍湯欲有明顯的抑制效果, 而麻杏甘石湯欲沒有抑制的效果, 而對 c-jun 則兩種藥皆無明顯的抑制效果, 在 cytokine 的方面, 麻杏甘石湯有輕微提高 TNF-alpha、IL-1beta 及 IL-10 之作用, 而小青龍湯無明顯作用, 對 TGF-beta 及 IL-13 兩藥皆無明顯的作用, 在 chemokines 上, 小青龍湯及麻杏甘石湯皆對 Eotaxin 有提升之現象, 但又以麻杏甘石湯較明顯, RANTES 之表現上小青龍湯有抑制表現的作用, 而麻杏石甘湯反而提升其表現, 而其餘尚有許多的基因要再進行分析, 找到有意義的目標, 再行 RT-PCR 確認。

#### 肆、討論

西方國家的氣喘盛行率日益增加, 因而發展出了衛生假說。此假說最主要的原則是認為新生兒時期的免疫系統是偏向於第二類輔助型 T 細胞, 需經過一段時間環境中適當的刺激, 才能產生平衡的免疫反應。會加強第一類輔助型 T 細胞反應而降低過敏發生率的因素有下列幾個: 肺結核分枝桿菌、麻疹病毒、B 型肝炎病毒的感染; 因接觸到較年長的兄弟姐妹而增加感染的機會; 出生六個月內曾待過日間照護中心; 減少環境中內毒素的暴露或降低內毒素接受體 (CD14) 的多型性因而導致  $\gamma$  型干擾素的減低。相反的, 若是常投予抗生素則會改變腸道菌種, 而阻礙了第一類和第二類輔助型 T 細胞之間的平衡。

在過敏性發炎反應中, Th2 細胞的角色不僅僅限於它能誘導 B 細胞製造過敏原特異性 IgE, 它亦能促進靶的組織嗜酸性白血球浸潤, 近來有些資料認為缺少 IgE 反應, 亦可能發生過敏反應, 事實上, B 細胞、IgE、CD40 及肥大細胞基因缺陷老鼠仍產生氣喘[36-39], 然而 CD4+T 細胞, IL-4, STAT6 及 IL-5 基因缺陷老鼠不能發生氣喘[40-43]。IL-4、IL-5 及 IL-13 能直接或間接解釋過敏患者大部份的病理生理上的症狀, IL-4 不但負責 IgE

isotypeswitching 同時亦負責循環中嗜酸性白血球粘附於內皮細胞[44]，嗜酸性白血球在 IL-5 及 eotaxin 的影響下被吸引至靶的組織[45]，再者，在 IL-4 或 IL-13 的控制下，eotaxin 由上皮細胞、內皮細胞及纖維細胞製造[46-48]，最後，IL-4、IL-9 及 IL-13 負責粘液細胞 (mucus cell) 粘液的過度分泌及誘導粘液細胞化生 (metaplasia) [49-50]。IL-4 及 IL-13 刺激纖維細胞生長和化學趨性以及合成細胞外基質蛋白質(extracellular matrix protein) [51-53]，而 IL-5、IL-9 則與上皮下纖維化 (subepithelial fibrosis)有關[54-58]。氣喘患者呈現 Th2 細胞增多的趨勢[16]，Th2 所分泌之細胞激素中 IL-4 能抑制 Th1 之活性，並能刺激 B 細胞產生 IgG1、IgE 抑制 IgG2a，調節 Th1 與 Th2 之間的平衡影響免疫過敏反應的程度，也影響氣喘反應中淋巴球之浸潤族群與細胞激素的分泌調節。因此，IL4 除了在 Th2 細胞分化上極為重要外，它在過敏性呼吸道疾病發生過程中還具有誘導 IgE 生成[17]，刺激肥大細胞的生長與發育[18]及誘導血管內皮細胞 VCAM-1 的表現[19]。同時，研究顯示暴露於過敏原時 IL-4 可媒介組織的嗜酸性白血球過多症[20]。相對的 Th1 細胞激素 IFN- $\gamma$ 、IL-10 對 Th2 細胞具負迴饋反應活性，降低呼吸道過度反應性、調降特異性 IgE 生成[16, 21-22]。在 IL-10 對免疫細胞之調控方面，人類的 Th1 與 Th2 細胞皆可分泌 IL-10，但是血液中的單核球及組織中的巨噬細胞可能是它最重要的來源。IL-10 可抑制 Th1 細胞產生非特異性前發炎細胞激素如 IL-1、IL-6、TNF- $\alpha$ 、IFN- $\gamma$  及 IL-2。此外，透過產生上述細胞激素，能有效的抑制單核吞噬細胞與自然殺手細胞[13-19]；IL-10 並可抑制 Th2 細胞產生過敏發炎有關的細胞激素，如 IL-4、IL-5 的形成。有研究報告指出，IL-10 能增加 T-細胞對過敏原的耐受性、抑制嗜酸性白血球存活與 IgE 的合成 [23-25]。

近來有10種TLRs在人類中發現，其中以TLR-2及TLR-4對葛蘭氏陽性菌的 lipoproteins and lipoteichoic acid (LTA)及葛蘭氏陰性菌LPS為人體的innate immunity 反應有關。雖然TLRs細菌有關，近來發現TLRs之信號傳遞有調控 Th1的反應，更有多篇文章談論到TLRs調節TH1/TH2的平衡及趨向有關 (60-62)。TLR2的號傳遞可壓制MITE所誘發過敏體內的單核球IL-5及IL-13的合成，而TLR-2之作用可經innate應對過敏原產生調適反應，不論對mite過敏或不過敏者，其TLR 表現並無差異，而當TLR-2受到刺激時，便會對過敏反應產生影響，產生IL-10，抑制Th2的作用，而減少IL-4、IL-5及IL-13之分泌，便減少IgE產生(63-65)。本實驗結果可見TLR-2在Der-P誘發下並無加，而在小青龍湯刺激下TLR-2 RNA量增加，而麻杏甘石湯也有刺激TLR-2略微增加的趨勢，推論小青龍湯可能增加TLR-2的產量，而使Th2受到抑制，進而減少過敏反應的發生。

在生物的 innate immunity receptors除了Toll-like receptors，尚有CD14，



在氣喘的病因中也佔一個重要的因子。西方國家的氣喘盛行率日益增加，因而發展出了衛生假說。此假說最主要的原則是認為新生兒時期的免疫系統是偏向於第二類輔助型T細胞，需經過一段時間環境中適當的刺激，才能產生平衡的免疫反應。會加強第一類輔助型T細胞反應而降低過敏發生率的因素有下列幾個：肺結核分枝桿菌、麻疹病毒、B型肝炎病毒的感染；因接觸到較年長的兄弟姐妹而增加了感染的機會；出生六個月內曾待過日間照護中心；減少環境中內毒素的暴露或降低內毒素接受體(CD14)的多型性因而導致 $\gamma$ 型干擾素的減低。相反的，若是常投予抗生素則會改變腸道菌種，而阻礙了第一類和第二類輔助型T細胞之間的平衡(66)。在美國研究中，發現CD14(-159CT及TT genotype)與增加IgE有關及增加過敏性疾病的危險性(67),但也有研究指出wheezing的小朋友比沒有wheezing小朋友血清中的cd14比較少,暗示有保護作用(68)。但總結CD14的多型性與氣喘發有關的，而接觸的時間很重要，如果羊水中及乳汁的CD14的量是增加的，導致過敏性疾病的發生是增加的(66)。而本實驗得到Der-p會增加CD14的表現，小青龍湯會明顯的抑制CD14表現，而麻杏石甘湯也會輕微的抑制，小青龍湯可能是降低CD14而減少過敏發作的危險性。

從目前實驗結果看來，小青龍湯可能與過敏性氣喘之趨化激素抑制有明顯相關性。在過敏性炎症病理機轉中，RANTES (regulated upon activation, normal T-cell expressed and secreted) 在嗜酸性白血球的成熟、遷移與活化過程中扮演重要角色(69-71)。RANTES 屬於CC 趨化激素(CC chemokine)，在周邊嗜酸性白血球中受補體(C3b/iC3b)及迦瑪干擾素刺激而分泌。其他如T細胞上皮細胞及血小板也都會分泌RANTES，在發炎反應中它能誘發黏附分子的產生，並使發炎細胞包括嗜酸白血球、記憶T細胞、單核球及血小板聚集。小青龍湯可以抑制其表現，可見在過敏性氣喘之趨化激素上小青龍湯治療佔有重要的地位。

抗發炎的細胞激素(可能是種限制發炎反應及發炎範圍的細胞激素，如IL-4, IL-10, IL-12)，這些抗發炎激素欲不會干擾對人體有益的宿主本身的第一線的防禦系統(如Defensin)及免疫作用(補體)(72)。而麻杏石甘湯可能是透過增加anti-inflammatory cytokine, IL-10之表現來限制過敏性氣喘之發炎作用來減緩熱性氣喘所產生急性發炎的現象。

由上述資料看來，寒藥麻杏石甘湯對過敏性氣喘之發炎作用之初期之限制發炎的程度是有所貢獻的，而熱藥小青龍湯阻止過敏性氣喘之遲發性期的嗜酸性白血球、記憶T細胞、單核球及血小板聚集，對氣喘遲發性期的治療有所成效；雖然本實驗之資料已有可解析寒熱藥對氣喘治療之成果，但我們認為應該還有不同的方向可以來詮釋寒熱藥之作用，我們正在努力的抽絲剝

菌，以期有更深入的發現；在解開中藥治氣喘的漫長道路上，我們最終的目的，希望先為中醫的寒熱藥對過敏性氣喘治療上在主觀的判斷之外，加入客觀的憑據，試圖為明確定義中醫寒熱藥治療之不同成效作出努力。同時，這項研究也具有運用於臨床療效評估的價值。並希望將目前研究基因最有利的技術---生物晶片，應用在中醫治療理論的研究，為傳統中醫理論研究邁入基因時代跨出一小步。

## 伍 結論與建議

熱藥小青龍湯可調控的基因表現遠比寒藥麻杏石甘湯多，而小青龍湯似乎對本氣喘鼠模式療效較佳，其調控的基因(如 TLR-2 或 CD14、NF-kB、C-fos)的表現，遠比寒藥麻杏石甘湯明顯；而目前看到中藥對 TLR-2 或 CD14 的調控，可以調節 Th1/Th2 的走向，是否是中藥治療氣喘有效的路徑，尚不確定，但 TLR-2 或 CD14 的調控是其中一環，這是不可否認的。

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## 柒、圖、表

Figure 2

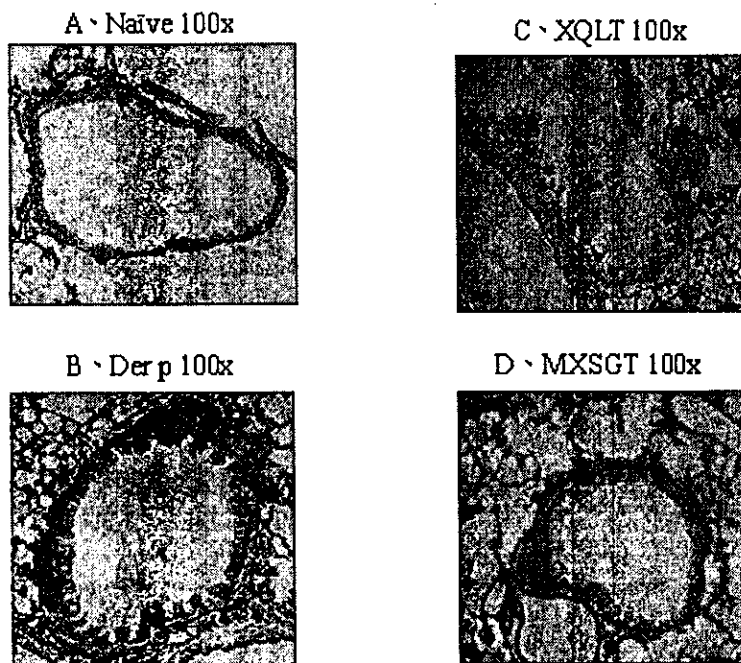


Figure2 各組小鼠肺部組織病理切片圖 (H&E stain 100X)

取小鼠左肺作肺組織切片染 H&E stain，觀察肺部發炎細胞浸潤及氣管壁增厚情形。

A 為 naive 組，B 為 Der p 組，C 為 XQLT 組，D 為 MXSGT 組。

Table 2

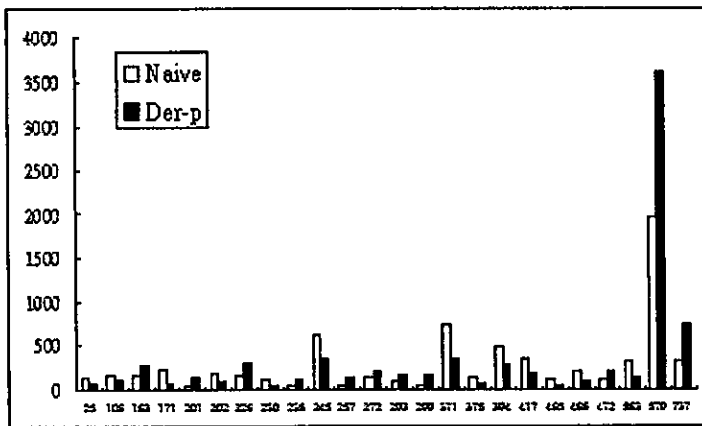
Table 1 各組小鼠 Der p 激發後 72 小時肺泡沖洗液中發炎細胞數目之變化

	Total cells	Macrophage	Lymphocytes (x 10 <sup>4</sup> )	Neutrophils	Eosinophils
Naïve	18.3±2.8	17.9±2.7	0.06±0.001	0.25±0.11	0
Der p	64.7±9.6*	49.8±1.2*	5.98±0.09*	11.2±0.91*	1.53±0.13*
Der p + XQLT	45.3±9.1	35.8±6.8	2.21±0.74#	6.63±1.9#	0.66±0.28#
Der p + MXSGT	32.1±7.6#	27.5±8.1#	1.23±0.25#	2.85±0.68#	0.40±0.19#

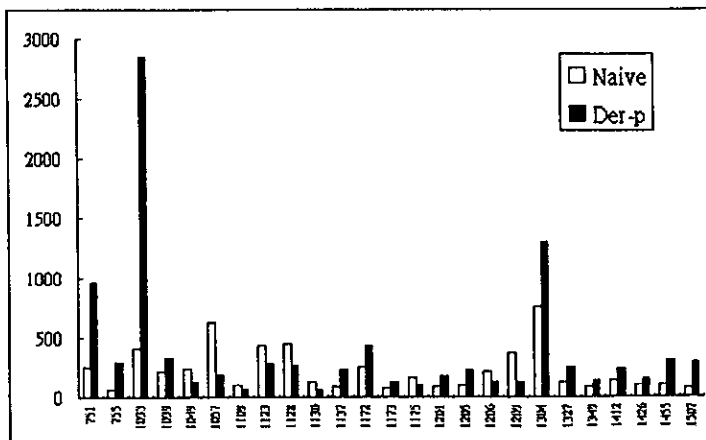
結果以 mean±SEM 表示。\*p<0.05，Der p 組與 naïve 組比較。#p<0.05，XQLT 或 MXSGT 組與 Der p 組比。XQLT:小青龍湯，MXSGT:麻杏石甘湯

Figure 3 Naïve 與 Der P 組 microarray 基因分析基因表現>1.5 倍與基因表現<0.5 倍者

A.

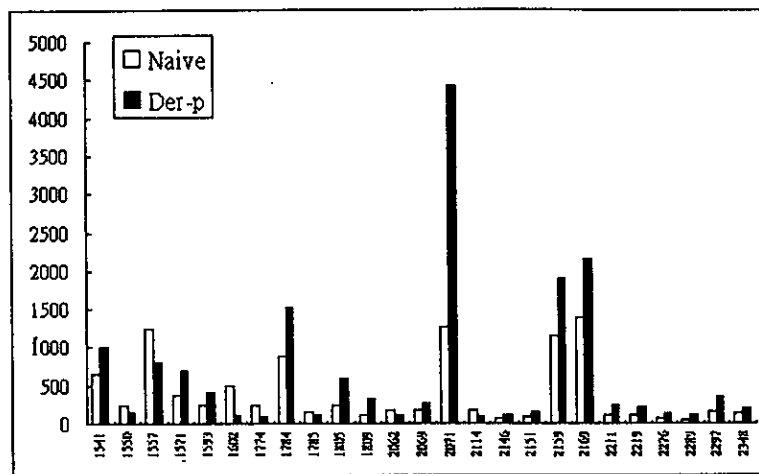


B.





C.



D.

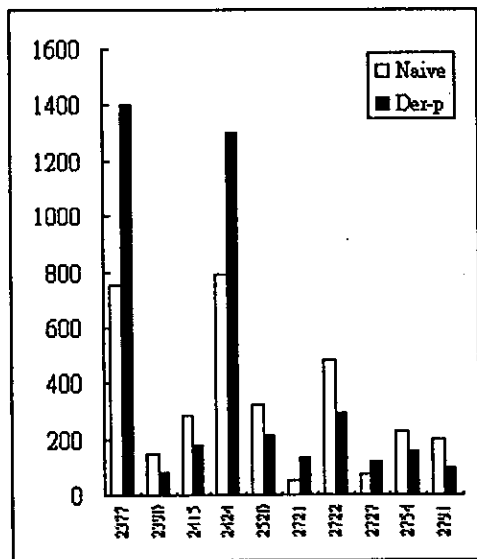


Table 3 DerP 組與 XQLT 組有顯著差異之基因

Gene list			
Index	Gene_id	Gene_note	Gene_id_1
2709	ENSMUSG00000040998	nephronectin (Npnt)	NM_033525
3211	ENSMUSG00000043165	loricrin (Lor)	NM_008508
17354	ENSMUSG00000032601	"protein kinase, cAMP dependent regulatory, type II alpha (Prkar2a)"	NM_008924
31755	ENSMUSG00000052003	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (Hmgcs1)	NM_145942
26585	ENSMUSG00000052974	"cytochrome P450, family 2, subfamily f, polypeptide 2 (Cyp2f2)"	NM_007817

14187	ENSMUSG00000013698	phosphoprotein enriched in astrocytes 15 (Pea15)	NM_011063
34705	ENSMUSG00000040998	nephronectin (Npnt)	NM_033525
590	Lutheran blood group (Auburger b antigen included) (Lu) NM_020486		
6543	ENSMUSG00000028426	RAD23b homolog (S. cerevisiae) (Rad23b)	NM_009011
19455	ENSMUSG00000030739	"myosin, heavy polypeptide 14 (Myh14)"	NM_028021
32203	ENSMUSG00000021156	"zinc finger, MYND domain containing 11 (Zmynd11)"	
	NM_144516		
3797	RIKEN cDNA 1700001F09 gene (1700001F09Rik)		NM_027940
26962	ENSMUSG00000061337	PREDICTED: similar to glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) (EC 1.2.1.12) - mouse (LOC435305)	XM_487217
18579	ENSMUSG00000025132	Rho GDP dissociation inhibitor (GDI) alpha (Arhgdia)	NM_133796
22458	ENSMUSG00000027076	translocase of inner mitochondrial membrane 13 homolog a (yeast) (Timm13a)	NM_013899
18145	ENSMUSG00000062019	heat shock protein 8 (Hspa8)	NM_031165
29022	ENSMUSG00000057789	BCL2-antagonist/killer 1 (Bak1)	NM_007523
34655	ENSMUSG00000029478	nuclear receptor co-repressor 2 (Ncor2)	NM_011424
31557	ENSMUSG00000006169	PREDICTED: expressed sequence AI642036 (AI642036)	
	XM_109767		
7266	ENSMUSG00000011884	glycolipid transfer protein (Gltp)	NM_019821
946	ENSMUSG00000025234	ariadne ubiquitin-conjugating enzyme E2 binding protein homolog 1 (Drosophila) (Arih1)	NM_019927
4225	ENSMUSG00000025223	LIM domain binding 1 (Ldb1)	NM_010697
18016	ENSMUSG00000044786	zinc finger protein 36 (Zfp36)	NM_011756
26834	ENSMUSG00000051660	PREDICTED: similar to sentrin 15 (LOC208231)	
	"XM_156206, XM_285829"		
17388	MAX gene associated (Mga)		NM_013720
34034	ENSMUSG00000052460	thyroid hormone receptor associated protein 3 (Thrap3)	NM_146153
20610	suprabasin (Sbsn)		NM_172205
22598	ENSMUSG00000048758	ribosomal protein L29 (Rpl29)	NM_009082
7453	ENSMUSG00000029328	heterogeneous nuclear ribonucleoprotein D-like (Hnrpdl)	NM_016690
9659	ENSMUSG00000019087	"ATPase, H transporting, lysosomal accessory protein 1 (Atp6ap1)"	
	NM_018794		
1800	ENSMUSG00000019820	utrophin (Utrn)	NM_011682
15363	ENSMUSG00000030579	TYRO protein tyrosine kinase binding protein (Tyrobp)	NM_011662
9961	ENSMUSG00000022698	Mak3 homolog (S. cerevisiae) (Mak3)	NM_028108
177	ENSMUSG00000001497	paired box gene 9 (Pax9)	NM_011041
34589	ENSMUSG00000002981	cleft lip and palate associated transmembrane protein 1 (Clptm1)	
	NM_019649		

35492	ENSMUSG00000038859	RIKEN cDNA 1300006M19 gene (1300006M19Rik)	
	NM_025833		
4194	ENSMUSG00000015340	"cytochrome b-245, beta polypeptide (Cybb)"	NM_007807
24578	ENSMUSG00000028127	"ATP-binding cassette, sub-family D (ALD), member 3 (Abcd3)"	
	NM_008991		
30838	ENSMUSG00000052212	RIKEN cDNA 1190003K14 gene (1190003K14Rik)	NM_026862
38205	ENSMUSG00000047729	RAS-related protein-1a (Rap1a)	NM_145541
32057	ENSMUSG00000031446	cullin 4A (Cul4a)	NM_146207
20206	ENSMUSG00000060002	choline phosphotransferase 1 (Chpt1)	NM_144807
13962	ENSMUSG00000021020	signal recognition particle 54 (Srp54)	NM_011899
18159	ENSMUSG00000057718	PREDICTED: similar to glyceraldehyde-3-phosphate dehydrogenase	
	(phosphorylating) (EC 1.2.1.12) - mouse (LOC435171)	XM_487067	
25741	ENSMUSG00000026421	cysteine and glycine-rich protein 1 (Csrp1)	NM_007791
4354	ENSMUSG00000007891	cathepsin D (Ctsd)	NM_009983
13782	ENSMUSG00000025013	tolloid-like 2 (Tll2)	NM_011904
34430	ENSMUSG00000059824	D site albumin promoter binding protein (Dbp)	NM_016974
37057	ENSMUSG00000031799	tropomyosin 4 (Tpm4)	NM_001001491
31286	ENSMUSG00000030990	FGF receptor activating protein 1 (Frag1)	NM_145583
17696	ENSMUSG00000041740	ring finger protein 10 (Rnf10)	NM_016698
16192	ENSMUSG00000007815	"ras homolog gene family, member A (Rhoa)"	NM_016802
16092	ENSMUSG00000020585	lysosomal-associated protein transmembrane 4A (Laptm4a)	NM_008640
36457	ENSMUSG00000055148	Kruppel-like factor 2 (lung) (Klf2)	NM_008452
35417	ENSMUSG00000024130	"ATP-binding cassette, sub-family A (ABC1), member 3 (Abca3)"	
	NM_013855		
2780	ENSMUSG00000030163	"C-type (calcium dependent, carbohydrate recognition domain) lectin, superfamily member 12 (Clecsf12)"	NM_020008
33027	ENSMUSG00000025017	phosphoinositide-3-kinase adaptor protein 1 (Pik3ap1)	NM_031376
27070		PREDICTED: RIKEN cDNA 2310026P19 gene (2310026P19Rik)	XM_126150
16209	ENSMUSG00000022125	"PREDICTED: ceroid-lipofuscinosis, neuronal 5 (Cln5)"	XM_127882
16272	ENSMUSG00000033904	RIKEN cDNA 6330503K22 gene (6330503K22Rik)	NM_182995
359	ENSMUSG00000020179	P lysozyme structural (Lzp-s)	NM_013590
14883	ENSMUSG00000058655	eukaryotic translation initiation factor 4B (Eif4b)	NM_145625
32411	ENSMUSG00000045414	PREDICTED: RIKEN cDNA 1190002N15 gene (1190002N15Rik)	
	XM_147036		
31455	ENSMUSG00000029810	RIKEN cDNA 1810009M01 gene (1810009M01Rik)	
	NM_023056		
25867	ENSMUSG00000034165	cyclin D3 (Cnd3)	NM_007632
24812	ENSMUSG00000029234	TPA regulated locus (Tparl)	NM_011626

5886 ENSMUSG00000036644 PREDICTED: RIKEN cDNA 2700008N14 gene (2700008N14Rik)  
XM\_109880

33380 "ENSMUSG00000059647,ENSMUSG00000029836,ENSMUSG00000057886" chromobox  
homolog 3 (Drosophila HP1 gamma) (Cbx3) NM\_007624

17086 ENSMUSG00000064057 "secretoglobin, family 3A, member 1 (Scgb3a1), transcript variant A"  
"NM\_054037,NM\_170727"

11433 ENSMUSG00000059588 calcitonin receptor-like (Calcr1) NM\_018782

5660 ENSMUSG00000025150 carbonyl reductase 2 (Cbr2) NM\_007621

2613 ENSMUSG00000021266 tryptophanyl-tRNA synthetase (Wars) NM\_011710

843 ENSMUSG00000030890 integrin linked kinase (Ilk) NM\_010562

26619 ENSMUSG00000022947 carbonyl reductase 3 (Cbr3) NM\_173047

25170 ENSMUSG00000026176 "CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A)  
small phosphatase 1 (Ctdsp1)" NM\_153088

9259 ENSMUSG00000054986 PREDICTED: similar to SEC14-like protein 3 (45 kDa secretory protein)  
(rsec45) (LOC380683) XM\_354597

26632 ENSMUSG00000025162 "casein kinase 1, delta (Csnk1d), transcript variant 2"  
"NM\_027874,NM\_139059"

594 ENSMUSG00000032548 "solute carrier organic anion transporter family, member 2a1 (Slco2a1)"  
NM\_033314

24090 ENSMUSG00000052440 PREDICTED: RIKEN cDNA 1500034J01 gene (1500034J01Rik)  
XM\_356085

30755 ENSMUSG00000016481 complement receptor related protein (Crry) NM\_013499

2655 ENSMUSG00000029467 "ATPase, Ca transporting, cardiac muscle, slow twitch 2 (Atp2a2)"  
NM\_009722

374 ENSMUSG00000028648 NADH dehydrogenase (ubiquinone) Fe-S protein 5 (Ndufs5)  
NM\_134104

33152 ENSMUSG00000050335 "lectin, galactose binding, soluble 3 (Lgals3)" NM\_010705

30705 ENSMUSG00000040462 RIKEN cDNA 4632413K17 gene (4632413K17Rik) NM\_177614

29333 ENSMUSG00000064193 "PREDICTED: similar to enolase 1, alpha non-neuron; alpha-enolase;  
2-phospho-D-glycerate hydrolase (LOC238943)" XM\_138902

17626 ENSMUSG00000021390 osteoglycin (Ogn) NM\_008760

2674 ENSMUSG00000034424 RIKEN cDNA 5730591C18 gene (5730591C18Rik) NM\_026572

30132 ENSMUSG00000064118 PREDICTED: similar to glyceraldehyde-3-phosphate dehydrogenase  
(phosphorylating) (EC 1.2.1.12) - mouse (LOC223365) XM\_122818

1940 ENSMUSG00000049866 ADP-ribosylation factor-like 7 (Arl7) NM\_177305

2557 ENSMUSG00000022564 "glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein  
1 (glutamate binding) (Grina)" NM\_023168

18673 ENSMUSG00000054452 amino-terminal enhancer of split (Aes) NM\_010347

35259	ENSMUSG00000001552	junction plakoglobin (Jup)	NM_010593
36182	ENSMUSG000000025647	scotin gene (Scotin)	NM_025858
30657	ENSMUSG000000031790	matrix metalloproteinase 15 (Mmp15)	NM_008609
17625	ENSMUSG000000020027	suppressor of cytokine signaling 2 (Socs2)	NM_007706
16115	ENSMUSG000000016087	Friend leukemia integration 1 (Fli1)	NM_008026
1040	ENSMUSG000000026739	B lymphoma Mo-MLV insertion region 1 (Bmi1)	NM_007552
31338	ENSMUSG000000040564	apolipoprotein C-I (ApoC1)	NM_007469
4591	ENSMUSG000000019978	erythrocyte protein band 4.1-like 2 (Epb4.1l2)	NM_013511
32274	ENSMUSG000000035770	PREDICTED: expressed sequence AA409702 (AA409702)	
	XM_134573		
32016	ENSMUSG000000031353	retinoblastoma binding protein 7 (Rbbp7)	NM_009031
36116	ENSMUSG000000037736	RIKEN cDNA 3732412D22 gene (3732412D22Rik)	
	NM_001001980		
20123	ENSMUSG000000018648	dual specificity phosphatase 14 (Dusp14)	NM_019819
26623	ENSMUSG000000022961	"Son cell proliferation protein (Son), transcript variant 1"	
	NM_178880		
1726	ENSMUSG000000010110	syntaxin 5A (Stx5a)	NM_019829
36920	ENSMUSG000000028651	peptidylprolyl isomerase E (cyclophilin E) (Ppie)	NM_019489
2575	ENSMUSG000000023243	"potassium channel, subfamily K, member 5 (Kcnk5)"	
	NM_021542		
3780		"transducin-like enhancer of split 3, homolog of Drosophila E(spl) (Tle3)"	NM_009389
30813	ENSMUSG000000044268	RIKEN cDNA 3010021M21 gene (3010021M21Rik)	
	NM_180600		
34948	ENSMUSG000000057188	PREDICTED: similar to RIKEN cDNA 3300002I08 (LOC433513)	
	XM_485110		
34178	ENSMUSG000000015522	aryl hydrocarbon receptor nuclear translocator (Arnt)	NM_009709
24581	ENSMUSG000000055053	nuclear factor I/C (Nfic)	NM_008688
32426	ENSMUSG000000050989	"selenoprotein N, 1 (Sepn1)"	NM_029100
32078	ENSMUSG000000023827	"1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, delta) (Agpat4)"	
	NM_026644		
181	ENSMUSG000000001588	"centaurin, beta 1 (Centb1)"	NM_153788
15327	ENSMUSG000000027775	RIKEN cDNA 1200003O06 gene (1200003O06Rik)	
	NM_025813		
15252	ENSMUSG000000024974	chondroitin sulfate proteoglycan 6 (Cspg6)	NM_007790
34652	ENSMUSG000000029560	sorting nexin 8 (Snx8)	NM_172277
22428	ENSMUSG000000030779	retinoblastoma binding protein 6 (Rbbp6)	NM_011247
29851	ENSMUSG000000063694	"cytochrome c, somatic (Cycs)"	
	NM_007808		
30420	ENSMUSG000000020923	"upstream binding transcription factor, RNA polymerase I (Ubtf)"	
	NM_011551		

24943	ENSMUSG00000063870	chromodomain helicase DNA binding protein 4 (Chd4)
	NM_145979	
26409	ENSMUSG00000001281	integrin beta 7 (Itgb7) NM_013566
17996	ENSMUSG000000038702	PREDICTED: RIKEN cDNA 9330132E09 gene (9330132E09Rik)
	XM_283635	
28983	ENSMUSG00000000948	small nuclear ribonucleoprotein N (Snrpn) NM_013670
30784	ENSMUSG000000032093	"CD3 antigen, epsilon polypeptide (Cd3e)" NM_007648
35487	ENSMUSG000000037221	motile sperm domain containing 3 (Mospd3) NM_030037
16027	ENSMUSG000000023927	special AT-rich sequence binding protein 1 (Satb1) NM_009122
31213	ENSMUSG000000024526	"cell death-inducing DNA fragmentation factor, alpha subunit-like
	effector A (Cidea)" NM_007702	
34409	ENSMUSG000000002015	B-cell receptor-associated protein 31 (Bcap31) NM_012060
18850	ENSMUSG000000054080	PREDICTED: similar to mastermind-like 2; mastermind
	(Drosophila)-like 2 (LOC434635)	XM_486495
34538	ENSMUSG000000016619	nucleoporin 50 (Nup50) NM_016714
36841	ENSMUSG000000030742	linker for activation of T cells (Lat) NM_010689
30952	ENSMUSG000000029881	PREDICTED: similar to T cell antigen receptor (LOC381765)
	XM_355761	
33205	ENSMUSG000000039982	deltex 4 homolog (Drosophila) (Dtx4) NM_172442
25012	ENSMUSG000000021975	"DNA segment, Chr 14, ERATO Doi 231, expressed (D14Erd231e)"
	NM_153414	
25	ENSMUSG000000032094	"CD3 antigen, delta polypeptide (Cd3d)" NM_013487
19440	ENSMUSG000000027852	neuroblastoma ras oncogene (Nras) NM_010937
8949	ENSMUSG000000015837	sequestosome 1 (Sqstm1) NM_011018
12090	ENSMUSG000000002320	transmembrane 9 superfamily member 1 (Tm9sf1) NM_028780
220	ENSMUSG000000024673	"membrane-spanning 4-domains, subfamily A, member 1 (Ms4a1)"
	NM_007641	
18154	ENSMUSG000000058147	X-linked lymphocyte-regulated 3b (Xlr3b) NM_011727
31226	ENSMUSG000000051217	RIKEN cDNA A630038E17 gene (A630038E17Rik) NM_172602
33128	ENSMUSG000000045092	endothelial differentiation sphingolipid G-protein-coupled receptor 1
	(Edg1) NM_007901	
34486	ENSMUSG000000041939	mevalonate kinase (Mvk) NM_023556
1252	ENSMUSG000000053977	"PREDICTED: CD8 antigen, alpha chain (Cd8a)" XM_132621

Table 4 Der p 組與 MXSGT 組有顯著差異之基因

Gene LIST			
Index	Gene_id	Gene_note	Gene_id_1
36457	ENSMUSG00000055148		Kruppel-like factor 2 (lung) (Klf2) NM_008452
6543	ENSMUSG00000028426		RAD23b homolog (S. cerevisiae) (Rad23b) NM_009011
29381	"ENSMUSG00000056954,ENSMUSG00000062933" peptidylprolyl isomerase A (Ppia)		
	"NM_008907, XM_356177, XM_485997"		
20610		suprabasin (Sbsn)	NM_172205
3797		RIKEN cDNA 1700001F09 gene (1700001F09Rik)	NM_027940
18673	ENSMUSG00000054452		amino-terminal enhancer of split (Aes) NM_010347
4225	ENSMUSG00000025223		LIM domain binding 1 (Ldb1) NM_010697
15389	ENSMUSG00000002944		CD36 antigen (Cd36) NM_007643
590		Lutheran blood group (Auberger b antigen included) (Lu)	NM_020486
8369	ENSMUSG00000028108		extracellular matrix protein 1 (Ecm1) NM_007899
33224	ENSMUSG00000046330		
31421	ENSMUSG00000052305		PREDICTED: hemoglobin beta chain complex (Hbb) XM_489729
3633	ENSMUSG00000052146		ribosomal protein S10 (Rps10) NM_025963
29618	ENSMUSG00000024653		"secretoglobin, family 1A, member 1 (uteroglobin) (Scgb1a1)"
	NM_011681		
30838	ENSMUSG00000052212		RIKEN cDNA 1190003K14 gene (1190003K14Rik) NM_026862
33152	ENSMUSG00000050335		"lectin, galactose binding, soluble 3 (Lgals3)" NM_010705
25170	ENSMUSG00000026176		"CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A)
	small phosphatase 1 (Ctdsp1)" NM_153088		
22986		ribosomal protein S28 (Rps28)	NM_016844
19784	ENSMUSG00000056116		"histocompatibility 2, T region locus 22 (H2-T22)" NM_010397
20225	ENSMUSG00000024803		ankyrin repeat domain 1 (cardiac muscle) (Ankrd1) NM_013468
17964	ENSMUSG00000023167		"hemoglobin, beta adult major chain (Hbb-b1)" NM_008220
300	ENSMUSG00000040904		"DNA segment, Chr 11, Brigham & Women's Genetics 0434 expressed
	(D11Bwg0434e)" NM_173742		
19789		MHC (A.CA/J(H-2K-f) class I antigen (LOC56628)	NM_019909
34763	ENSMUSG00000021243		RIKEN cDNA 1110008B24 gene (1110008B24Rik) NM_028632
36128	ENSMUSG00000062169		PREDICTED: RIKEN cDNA E430023H19 gene (E430023H19Rik)
	XM_484947		
20547	ENSMUSG00000037079		PREDICTED: similar to uncharacterized hypothalamus protein HT013
	(LOC228730) XM_130476		
25867	ENSMUSG00000034165		cyclin D3 (Cnd3) NM_007632
35417	ENSMUSG00000024130		"ATP-binding cassette, sub-family A (ABC1), member 3 (Abca3)"
	NM_013855		

35744	ENSMUSG00000061613	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 1 (U2af1)
	NM_024187	
29057	ENSMUSG00000031839	heat shock factor binding protein 1 (Hsbp1) NM_024219
8441	ENSMUSG00000053475	tumor necrosis factor alpha induced protein 6 (Tnfaip6) NM_009398
6023	ENSMUSG00000046805	PREDICTED: macrophage expressed gene 1 (Mpeg1) XM_129176
26585	ENSMUSG00000052974	"cytochrome P450, family 2, subfamily f, polypeptide 2 (Cyp2f2)"
	NM_007817	
14187	ENSMUSG00000013698	phosphoprotein enriched in astrocytes 15 (Pea15) NM_011063
34705	ENSMUSG00000040998	nephronectin (Npnt) NM_033525
29046	ENSMUSG00000057530	endothelin converting enzyme 1 (Ece1) NM_199307
21791	ENSMUSG00000003617	ceruloplasmin (Cp) NM_007752
33625	ENSMUSG00000002033	"CD3 antigen, gamma polypeptide (Cd3g)" NM_009850
31392	ENSMUSG00000005683	citrate synthase (Cs) NM_026444
16821	ENSMUSG00000018819	lymphocyte specific 1 (Lsp1) NM_019391
36331	ENSMUSG00000018501	nuclear receptor co-repressor 1 (Ncor1) NM_011308
29158	ENSMUSG00000016319	"solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5 (Slc25a5)" NM_007451
35278	ENSMUSG00000025261	PREDICTED: upstream regulatory element binding protein 1e (Ureb1)
	XM_136108	
33128	ENSMUSG00000045092	endothelial differentiation sphingolipid G-protein-coupled receptor 1
	(Edg1) NM_007901	
16590	ENSMUSG00000054690	endomucin (Emcn) NM_016885
5184	ENSMUSG00000032011	"thymus cell antigen 1, theta (Thy1)" NM_009382



Figure 4

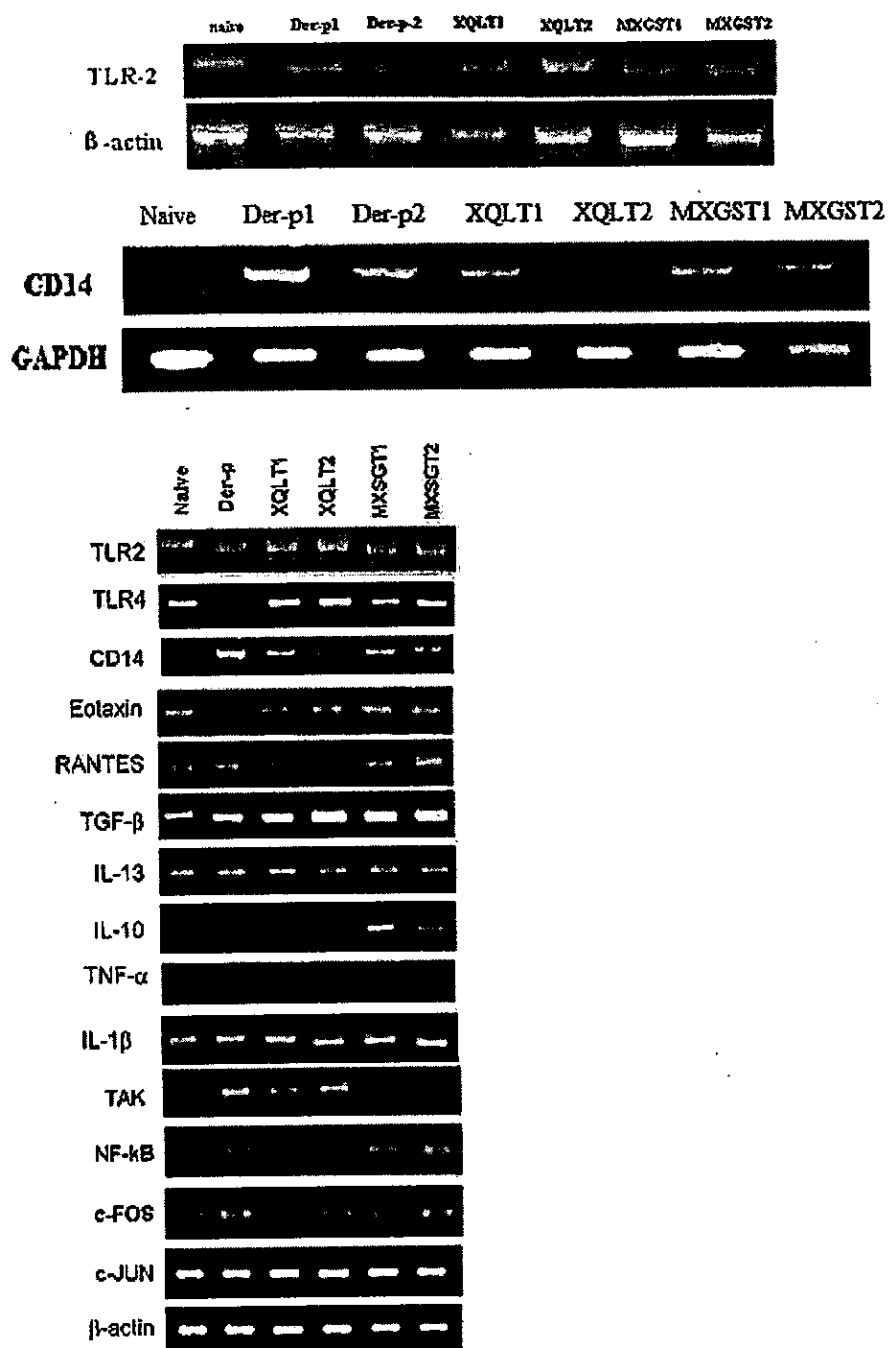


Fig4.以 RT-PCR 比較各組之變化