行政院國家科學委員會專題研究計畫 成果報告

以蛋白體學、RNA 干擾和免疫促進寡核酸技術探討魚類膠原蛋白引起之食品過敏機制
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2313-B-041-011-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：嘉南藥理科技大學生活應用與保健系

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 96 年 07 月 31 日

目　錄

INTRODUCTION

Due to the extremely increases of the worldwide prevalence of allergic diseases, the exact molecular mechanism of pathological or inflammatory allergy events as well as potential preventative or therapeutic strategies are getting increasingly importance. Mast cells and basophils are major effector cells in allergic progression through their highly expressed FcεRI on their cell surface. The activations on T cells, Th cells, B cells, mast cells and basophils are initiated once allergens cross the epithelial membrane. These events trigger preformed, synthetic and secreted of particular chemical mediators. Among them, IL-4, IL-5 and IL-13 are major cytokines for IgE production, facilitate to Th2 differentiation and allergic responses. Basophils are a dominant and rapid source not only for large amount of IL-4 and IL-13, but constitutive expression of CD40L and CCR3 on their surface. Basophils have been detected in particular organs affected by allergic reactions, such as bronchial and airway biopsies from asthma patients, and nasal lavage fluids from allergic rhinitis patients, as well as skin biopsies from atopic and contact dermatitis.

In the field of allergy diagnosis, basophil superior than mast cells are selected for in vivo and in vitro functional tests. Upon experience of specific allergen that brings up with membrane-bound IgE productions, basophils not only synthesize and release chemical mediators but heavily elevated the expression of certain activation markers (ie. CD45, CD63, CD69 and CD203c) that can be enumerated by flow cytometry. The CD63, the most commonly used markers in basophil activation test (BAT), is normally expressed on the vesicle membrane. On allergen-activated basophils, the fusion of the vesicle to the plasma membrane leads to highly expression of CD63. The BAT is broadly applied in examining IgE-mediated allergies including house dust mite allergy, inhalant allergy, primary and secondary (pollen-associated) food allergy, natural rubber latex allergy, Hymenoptera veom allergy, and several drugs as β-lactam antibiotics, muscle relaxants and metamizol.

With the advent of proteomics and allergen sequence, the study of allergome has become conceivable. The allergome can be defined as the characterization of allergens based on a proteomics approach. The biomic study of immunological molecular mechanisms will enable more rapid advances in elucidating allergic inflammatory pathways through proteomics methodologies coupled with bioinformatics techniques. Proteomics characterizes cellular proteins and as well as its abundance, state of modification, protein complexes and interactions. The global changes in cellular protein expression can be visualized by two-dimensional gel electrophoresis and identified by mass spectrometry analysis.

The phorbol 12-myristate 13-acetate (PMA), a structure analogous to diacylglycerol, can activate protein kinase C (PKC). The PKC is a key enzyme to degranulation, cytokine and chemokine production in mast cells and basophils. The calcimycin A23187 greatly increases the ability of divalent ions, highly selective for Ca^{2+}, to cross biological membranes. It is commonly used to elevate intracellular Ca^{2+} levels in intact cells. Since IL-4 and IL-13 that are central in allergic inflammation share several biological properties, it is crucial to elucidate how

human basophils synthesis and release such cytokines upon stimulation. The PMA induction of human basophils brings about IL-13 not IL-4 secretion. In contrast, the ionomycin only initiates IL-4 not IL-13 production. More recently, combinational use of PMA and A23187 for human basophils seems to be a representative effector cell model for allergic molecular mechanism, signaling pathway, transcriptional factor activation, and protective lead compound screening.

METHODS

Culture Medium

RPMI 1640 (Sigma-Aldrich, MO, USA) supplemented with 10% fetal bovine serum (Gibco BRL, NY, USA), 1.5 g/L sodium bicarbonate (Atlanta Biologicals, GA, USA), 2 mM L-glutamine, 1% antibiotic mixture, 4.5 g/L glucose, 10 mM HEPES, and 1 mM sodium pyruvate (Atlanta Biologicals, GA, USA) was used as the culture medium.

Chemicals

The phorbol 12-myristate 13-acetate (PMA) and A23187 were obtained from Sigma (MO, USA). PMA and A23187 were dissolved in dimethyl sulfoxide (DMSO). Control groups were incubated with an equal concentration of DMSO.

RT-PCR analysis of cytokine (IL-4, IL-5, IL-6, IL-8, IL-13, IL-1β, and IFN-γ) mRNA expression

KU812 cells (5×10^5 cells/mL) were first incubated without (equivalent amount of PBS) or with *G. lucidum* polysaccharides extracts (G4 and F3) at 100 μg/μl dosages for 30 minutes. Then PMA (10 ng/mL) plus A23187 (1 μmol/L) or an equivalent amount of diluted DMSO was added and further incubated for 3 hours. After washing, total RNA was extracted from the cell pellets by using the Trizol protocol (Invitrogn, Inc). Total RNA (800 ng) was then mixed with the RT mixture according to the manufacturer's protocol (Invitrogen) and incubated at 42°C for 50 minutes, followed by 95°C for 5 minutes. After each RT, PCR amplification was performed with the following primers. IL-4–sense, 5′ATG-GGT-CTCACC-TCC-CAA-CTG-CT 3′; antisense, 5′ GTT-TTC-CAA-CGT-ACT-CTG-GTT-GGC 3′. IL-5–sense, 5′ GCT-TCT-GCA-TTT-GAG-TTT-GCT-AGC-T 3′; antisense 5′ TGG-CCG-TCA- ATG-TAT-TTC-TTT-ATT-AAG 3′. IL-6–sense, 5′ ATG-AAC-TCC-TTC-TCC- ACA-AGC-GC 3′; antisense, 5′ GAA-GAG-CCC-TCA-GGC-TGG-ACT-G 3′. IL-8–sense, 5′ATG-ACT-TCC-AAG-CTG-GCC-GTG-GCT 3′; antisense, 5′ TCT- CAG-CCC-TCT-TCA-AAA-ACT-TCT-C 3′. IL-13–sense, 5′ CCA-CGG-TCA- TTG-CTC-TCA-CTT-GCC 3′; antisense, 5′ CCT-TGT-GCG-GGC-AGA-ATC-CGC-TCA3′. IL-1β–sense, 5′ ATG-GCA-GAA-GTA-CCT-AAG-CTC-GC 3′; antisense, 5′ ACA-CAA-ATTGCA-TGG-TGA-AGT-CAG-TT 3′. IFN-r–sense, 5′ TGT-TAC-TGC-CAG-GAC-CCA-TAT-GTA-AAA 3′, antisense, 5′ CAT-CAC- TTG-GAT-GAG-TTC- ATG-TAT-TGC 3. ′β-Actin–specific primers were as follows: sense, 5′ GTG-GGG-CGC-CCC-AGG-CAC-CA 3′; antisense,5′ GTC-CTT- AAT-GTC-ACG-CAC-GAT-TTC 3′. The PCR process was performed with a Thermo Px2Thermal Cycler. The products were electrophoresed in 2% agarose gel, stained with ethidium bromide, photographed, and quantitated by computer program.

ELISA analysis of IL-4 protein expression

KU812 cells (3×10^6 cells/mL) were incubated without (equivalent amount of diluted PBS) or with G4 and F3 at an indicated dose for 30 minutes and then stimulated with A23187 (1 μmol/L) plus PMA (10ng/mL) for 6 hours to induce maximal synthesis of IL-4. The supernatant was harvested, and the IL-4 protein level was measured by an ultrasensitive human IL-4 ELISA kit (Biosource International). The measurable range of IL-4 was 65 to 25,000 fg/mL.

Two dimensional (2-D) electrophoresis

Lyophilized proteins were fractionated solubilized in lysis buffer, consisting of 7 M urea, 2 mM thiourea, 4% CHAPS, 65 mM DTE, 2% ampholytes, and 0.0002% bromophenol blue. Extraction buffer was kept by centrifugation at 10 000×*g* for 10 min and reserved for running 2-D PAGE. The protein concentration was determined by a PlusOne protein assay kit (Amersham Biosciences, Uppsala, Sweden). The protein concentration of the supernatant was adjusted to approximately 500 μg/350 μL. 2-DE for each variety of samples was performed at least 3 times. Approximately 500 μg/350 μL lysis buffer of extracted proteins was loaded onto 18 cm IPG strips, pH range 3–10 (Amersham Biosciences). Strips were rehydrated in the presence of sample solution plus 0.5% ampholyte buffer under constant low voltage (50 V) for 12 h. The first dimensional isoelectric focusing (IEF) was conducted at 20℃ using an IPG-phoreII (Amersham Biosciences), programmed with voltages from 100 V for 3 h, 350 V for 1 h, 500 V for 1 h, 1000 V for 1 h, 5000 V for 1 h, and 8000 V for a total of 66 kVh. After the first-dimensional IEF, the strips were incubated for 20 min in equilibration buffer, containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS plus DTE, followed by incubation in equilibration buffer plus with iodoacetamide for 20 min. The second-dimensional electrophoresis using Protean II xi Multi-Cells (Bio-Rad Laboratories, Hercules, CA, USA) was performed on 12.5% linear polyacrylamide gels with 45 mA/gel until the buffer front line was 5–10mm from the bottom of the gel. Proteins were stained with Sypro Ruby. Analytical and preparative gels were fixed with 10% methanol and 7% acetic acid for 30 min. The gels were washed with ddH_2O and then stained with 500 mL of Sypro Ruby dye solution for 3 h. The developed gels were digitally scanned as 2D images using fluorescence image scanning Typhoon 9200 (Amersham Pharmacia Biotech), then analyzed using PDQuest software (BioRad, Hercules, CA, USA) to automatically detect and quantify protein spots. Intensity levels were normalized between gels using proportion of the total protein intensity detected for the entire gel.

In-gel Digestion

From the 2-D gel analysis of controlled and induced samples, proteins that expressed differently were selected for identification by mass spectroscopy. These spots were cut from 2D-gels, sliced into 1 mm^3 pieces, and then washed three times with 200 μl water and 50mM ammonium bicarbonate buffer (pH8.0) in 50% acetonitrile for 15 minutes. The gel pieces were dehydrated in 50 mM ammonium bicarbonate buffer (pH8.0) for 5 minutes, then added an equal volume of 100% acetonitrile. After 15 minutes of incubation, all liquid was removed. 100% acetonitrile was added again to cover the gel pieces. After 100% acetonitrile was removed, the gel pieces were dried down in a vacuum centrifuge. Enzyme digestion was performed by adding 15 μl trypsin in 25mM ammonium bicarbonate to a final concentration of 5 ng per sample at 56℃ for 1 hour. The peptides fragments were extracted with equal volume 100% acetonitrile/2% trifluoroacetic acid (TFA), sonicated in a bath for 10 minutes, and then the supernatant was recovered and collected in the new tube. The previous tube with gels slices was added 20 μl 50% acetonitrile/1% TFA, sonicated for 10 minutes and then the supernatant was recovered and collected in the previous new tube. We repeated the step above again. The extracted peptides were concentrated by centrifugation in a vacuum centrifuge, resolubilized with 5 μl of 50 % acetonitrile/ 1% TFA, and directly spotted on the sample plate of a MALDI-TOF mass spectrometry.

MALDI-TOF mass spectrometry

α-cyano-4-hydroxycinnamic acid (0.5 μl of 10 mg/ml) was applied to each spot, and thespots were air-dried at room temperature prior to acquiring mass spectra (MALDI^{TM}, Micromass, Manchester, UK). Monoisotopic peptide mass values were inputted for database search by Mascot (http://www.matrixscience.com/) against NCBInr and Swiss-Prot protein databases. Swiss-Prot Peptide mass mapping, a particularly successful method for the identification of proteins as described in the literature, was used to identify our proteins. Our protein selection

criteria are: a good match of at least five fragments from a single 2-D gel spot against a single protein sequence entry in the database, the high coverage value, and the human-origin sequence. Then this protein is considered as a candidate. MS/MS was performed using a MALDI-Q-TOF-MS hybrid quadrupole/orthogonal acceleration TOF spectrometer (MALDI™, Micromass, Manchester, UK). The product ion spectra generated by Q-TOF MS/MS were searched against NCBInr and Swiss-PROT databases for exact matches using the Mascot search program.

RESULTS AND DISCUSSION

The aims of this studies is to establish the first proteomics database of stimulated basophils after PMA plus A23187 stimulation by human basophil cell line KU812. With RT-PCR, the up-regulation of IL-4, IL-5, IL-6, IL-8, IL-13, and IFN-γ, but not for IL-1β were detected after PMA plus A23187 stimulation at 3 hours. Among them, IL-4, IL-5, and IFN-γ were apparently enhanced (Fig. 1). Three hours stimulation made these cytokines reach their maximum levels in our preliminary studies (data not shown).

At present, for 3 hr treatment, we identified 2 proteins including heterogeneous nuclear ribonucleoprotein K and A2/B1 type (hnRNP K and hnRNP A2/B1). For 6 hr treatment, PMA plus A23187 induced down-regulation of Protein SET, tubulin b-5 chain, stress 60 protein, ATP-dependent RNA helicase, actin a/b-chain, fascin, a-enolase, furmarate hydratase, 26S protease regulatory subunit63, Fructose-bisphosphate aldolase A/C, glyceraldehyde-3-phosphate dehydrogenase, profilin1 expressions. Three types of heterogeneous nuclear ribonucleoprotein (HNRPK, HNRPF, HNRPAB) were associated to PMA plus A23187 stimulated KU812 cell. The HNRPK and HNRPAB were not shown significant difference from 1-D western blot patterns.

The heat shock protein 70 family, consists of heat shock 70 kDa protein 1 (HSPA1A, P08107) and 75 kDa glucose-regulated protein (HSPA9B, P38646), were positively expression. Stress proteins are proved to possess dominant immuno-modulatory capability, both at the levels of innate immunity and antigen specific adaptive immunity. More recently, Toll-like receptors (TLR) were shown to have multiple ligands, among which are heat shock protein families. TLR4 and TLR2 revealed to be receptors for hsp60 and hsp70, respectively. Due to admirable immunogenicity of hsp family, hsp have been designed for their adjunct use in vaccines. In this study presented, stress-70 protein family in KU812 cells were down-regulated after PMA plus A23187 stimulation may point toward immunosuppressive mechanism raised by such stimulus. The biological function classification and their relationship to immunology or allergy is being investigated.

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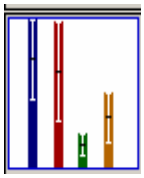
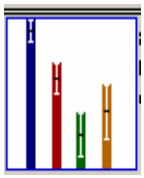
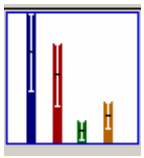
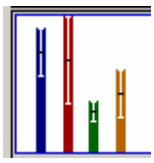
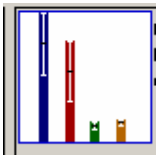
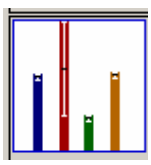
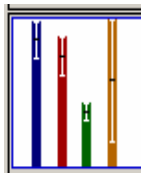
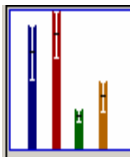
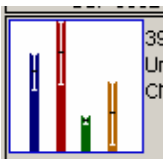
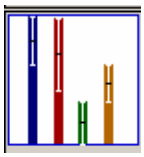
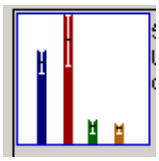
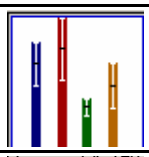
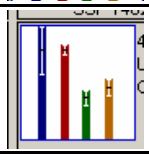
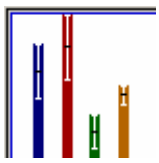
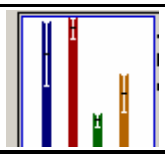
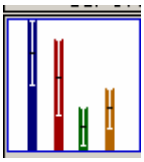
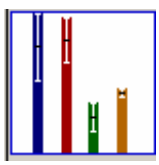
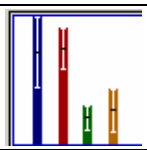
Table. 1. Summary of down-regulated proteins in total cell lysates of KU812 cells after 6 hr PMA+A23187 treatment by MALDI-Q-TOF analysis

Spot	Change	Accession	Protein name	Score	Theoretic		SC	Matched sequences
	Con 3h 6h 12h	Sprot			Mr (kD)	p*I*		
0502 (1)		Q01105 Hs. 436687	Protein SET	55	33.5	4.23	8	LRQPFFQK SGYRIDFYFDENPYFENK
1402 (3)		P46783 Hs. 645317	40S ribosomal protein SA	85	32.8	4.79	11	AIVAIENPADVSVISSR FTPGTFTNQIQAAFREPR
1503 (5)		P07437 Hs. 636480	Tubulin β-5 chain	211	49.6	4.78	10	ISVYYNEATGGKYVPR LHFFMPGFAPLTSR MAVTFIGNSTAIQELFKR
2604 (11)		P10809 Hs. 595053	Heat shock protein 60	214	61.0	5.70	6	TLNDELEIIEGMKFDR KISSIQSIVPALEIANAHR ISSIQSIVPALEIANAHR
2504 (12)		P52597 Hs. 808	Heterogeneous nuclear ribonucleoprotein F	76	45.6	5.38	4	ATENDIYNFFSPLNPVR
(13)		P60842 Hs. 129673	Eukaryotic initiation factor 4A-I	126	46.1	5.32	8	GIYAYGFEKPSAIQQR AEVQKLQMEAPHIIVGTPGR
2402 (14)		P60709 Hs. 520640	Actin β-chain	275	41.7	5.29	23	AVFPSIVGRPR VAPEEHPVLLTEAPLNPK LCYVALDFEQEMATAASSSSLEK SYELPDGQVITIGNER DLYANTVLSGGTTMYPGIADR
2507 (15)		P62736 Hs. 500483	Actin α-chain	57	42.0	5.23	4	SYELPDGQVITIGNER
3202 (16)		P47756 Hs. 432760	F-actin capping protein subunit beta	75	31.3	5.36	5	KLEVEANNAFDQYR

4502 (18)		P23381 Hs. 497599	Tryptophanyl-tRNA synthetase, cytoplasmic	126	53.1	5.83	7	ISFPAIQAAPSFSNSFPQIFR ALIEVLQPLIAEHQAR
4504 (19)		P06733 Hs. 517145	α-enolase	70	47.1	7.01	3	LAMQEFMILPVGAANFR
6601 (21)		P54577 Hs. 213264	Tyrosyl-tRNA synthetase, cytoplasmic	51	59.1	6.61	3	TVVSGLVQFVPKEELQDR
6502 (24)		P06733 Hs. 517145	α-enolase	258	47.1	7.01	12	EIFDSRGNPTVEVDLFTSK LAMQEFMILPVGAANFR AGYTDKVVIGMDVAASEFFR
7504 (25)		P07954 Hs. 592490	Furmarate hydratase	33	54.6	8.85	4	THTQDAVPLTLGQEFSGYVQQVK
6404 (26)		P62333 Hs. 156171	26S protease regulatory subunit	63	44.1	7.10	3	EVIELPLTNPELFQR
6402 (27)		P06733 Hs. 517145	α-enolase	67	47.1	7.01	8	LAMQEFMILPVGAANFR AGYTDKVVIGMDVAASEFFR
5406 (28)		Q6FH94 Hs. 155247	Fructose-bisphosphate aldolase C	75	39.4	6.41	4	TPSALAILENANVLAR
7403 (29)		P04075 Hs. 513490	Fructose-bisphosphate aldolase A	51	39.4	8.30	5	IGEHTPSALAIMENANVLAR
8302 (30)		P04406 Hs. 479728	Glyceraldehyde-3-phosphate dehydrogenase	128	36.0	8.57	11	VIHDNFGIVEGLMTTVHAITATQK LISWYDNEFGYSNR

8113 (33)		P07737 Hs. 494691	Profilin-1	126	15.0	8.44	12	TFVNITPAEVGVLVGK TFVNITPAEVGVLVGKDR

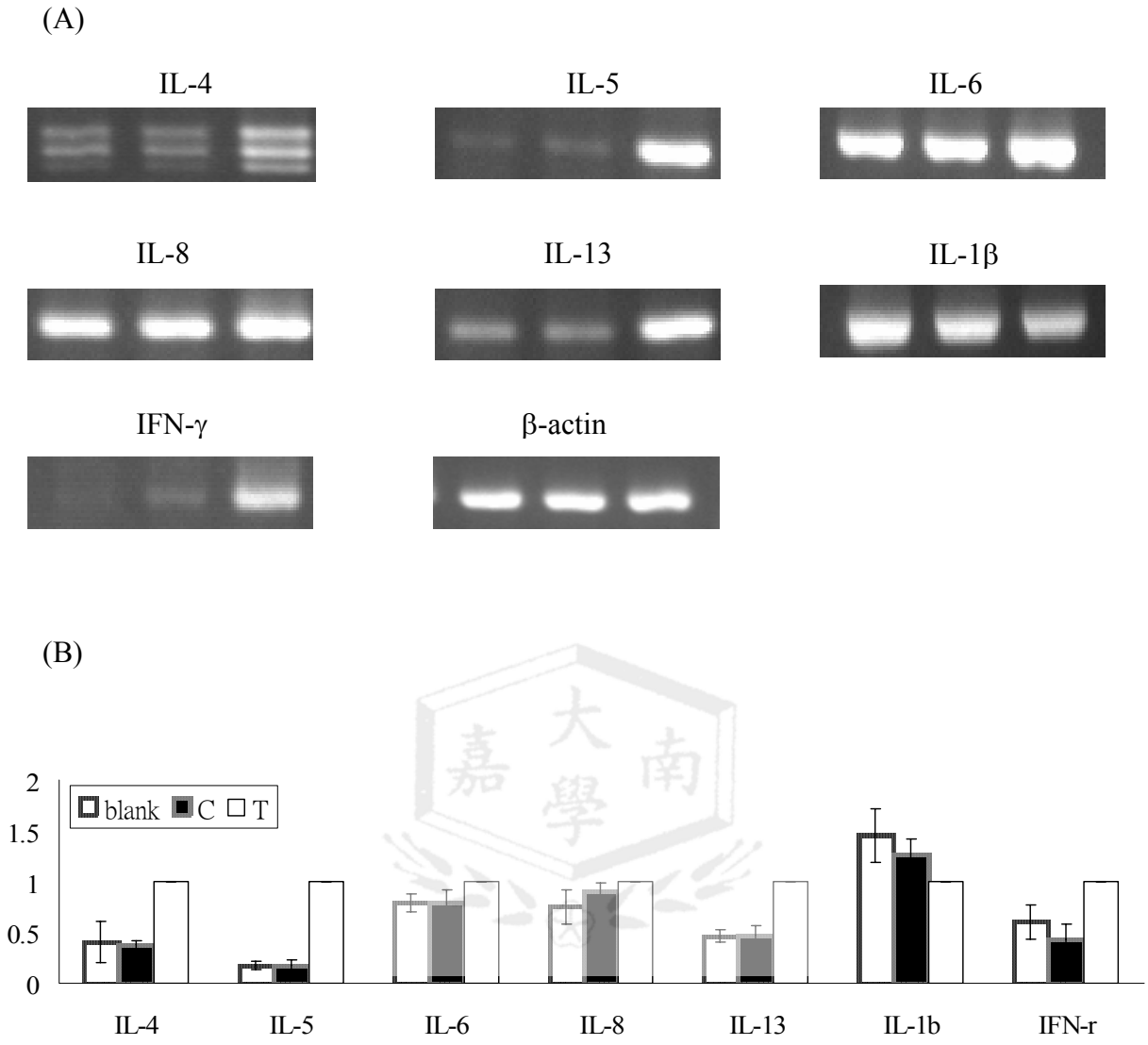


Fig. 1. RT-PCR patterns for cytokines of human basophil cell line KU812 after treated with PMA plus A23187. (A). RT-PCR patterns of IL-4, IL-5, IL-6, IL-8, IL-13, IL-1β, and IFN-γ. (B). Quantification profiles of IL-4, IL-5, IL-6, IL-8, IL-13, IL-1β, and IFN-γ by densitometry. The cells (5 x 10^5 cells/mL) were incubated with nothing or stimulated with PMA (10 ng/mL) plus A23187 (1 μmol/L) or an equivalent amount of diluted DMSO for 3 hours.

*Blank: treated with nothing; Con: only treated with an equivalent amount of diluted DMSO; Induced: stimulated with PMA plus A23187.

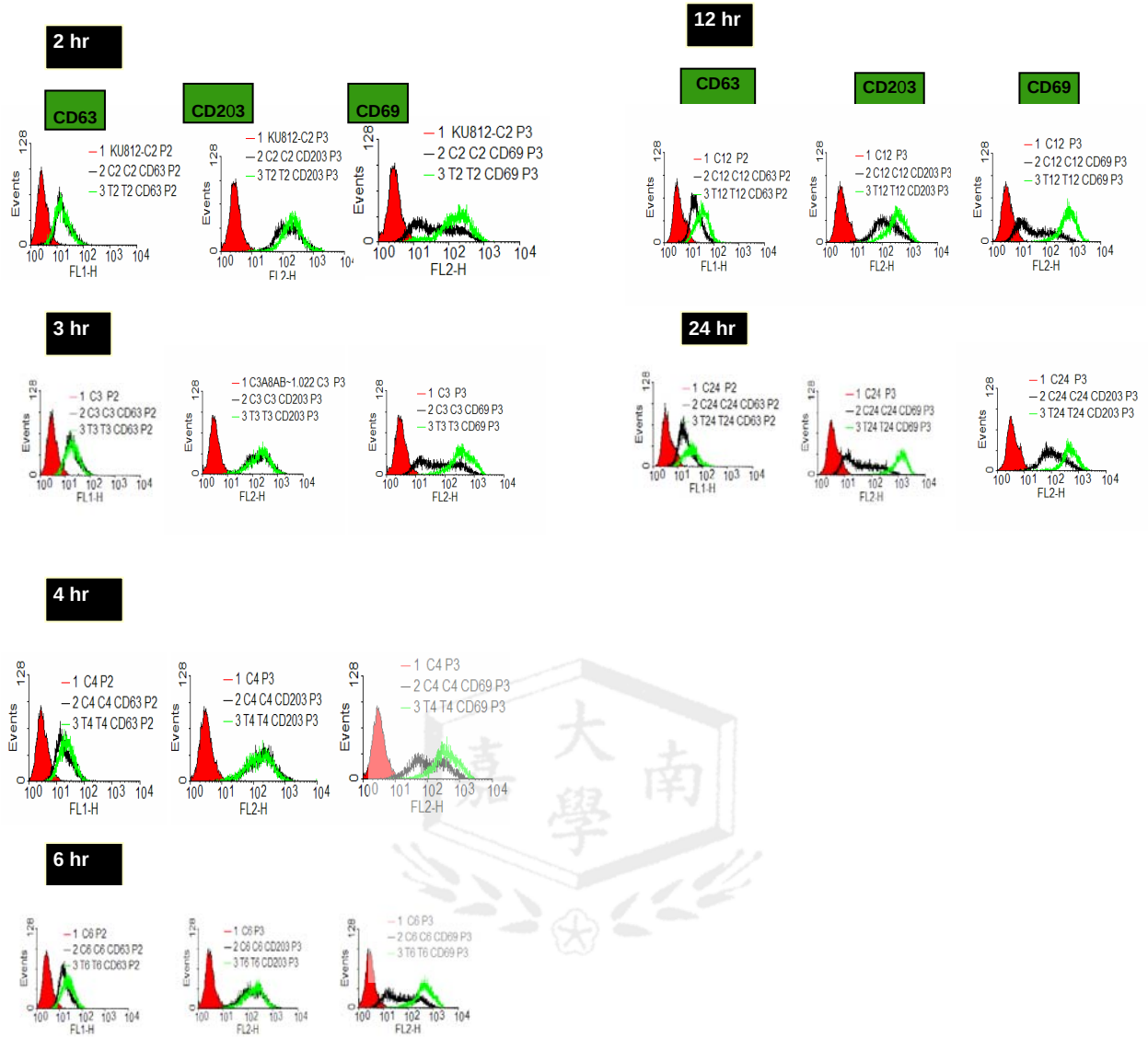


Fig. 2. Flow cytometry patterns for CD63, CD69, and CD203c expressions when stimulated with PMA plus A23187 at different time periods.

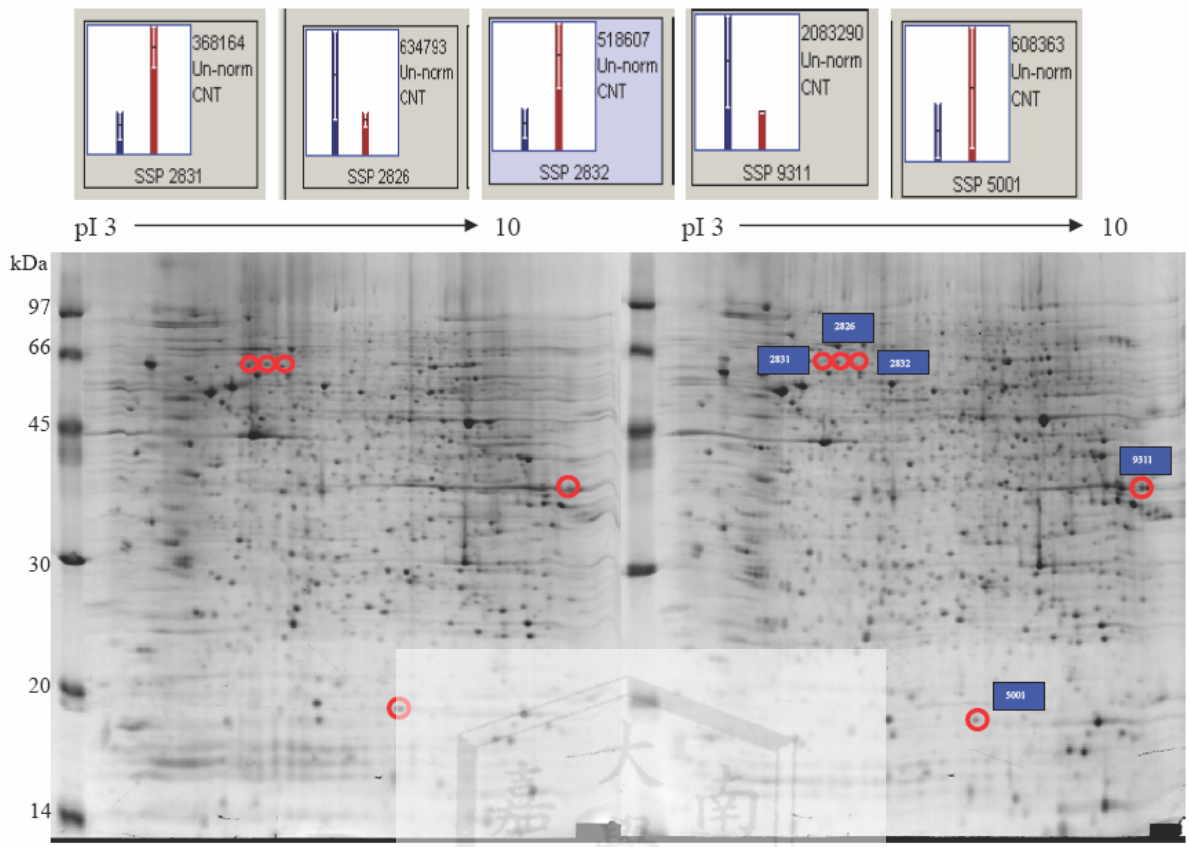


Fig. 3. Two-dimensional electrophoresis (2DE) patterns of human basophil cell line KU812 after treated with diluted DMSO and PMA plus A23187 for 3 hr.

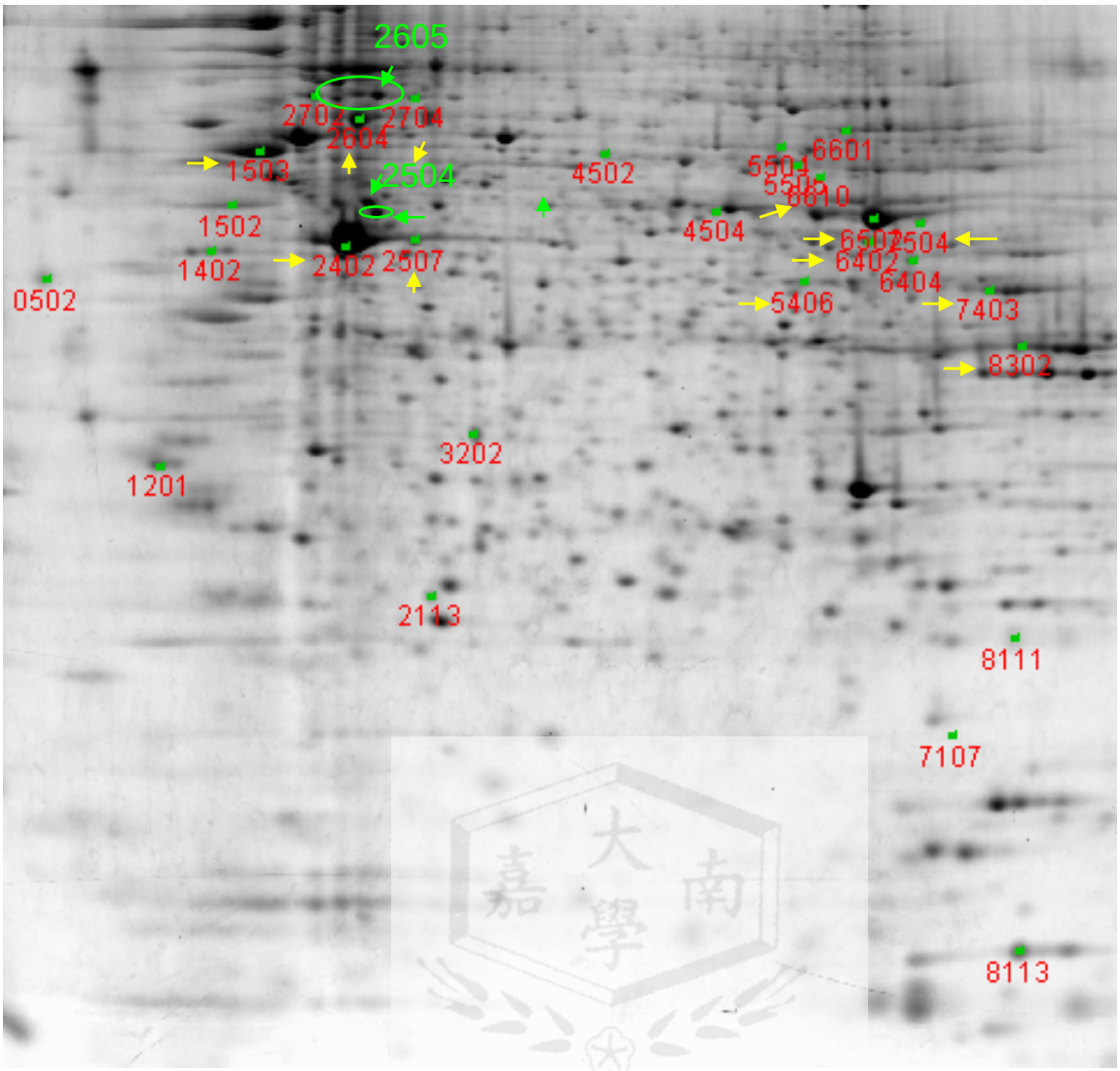


Fig. 4. The representative 2DE gel for 6 hr PMA+A23187 treatment. Protein spots present in regions in the pI range of 3-10 and molecular weight range of 14-97 kD.

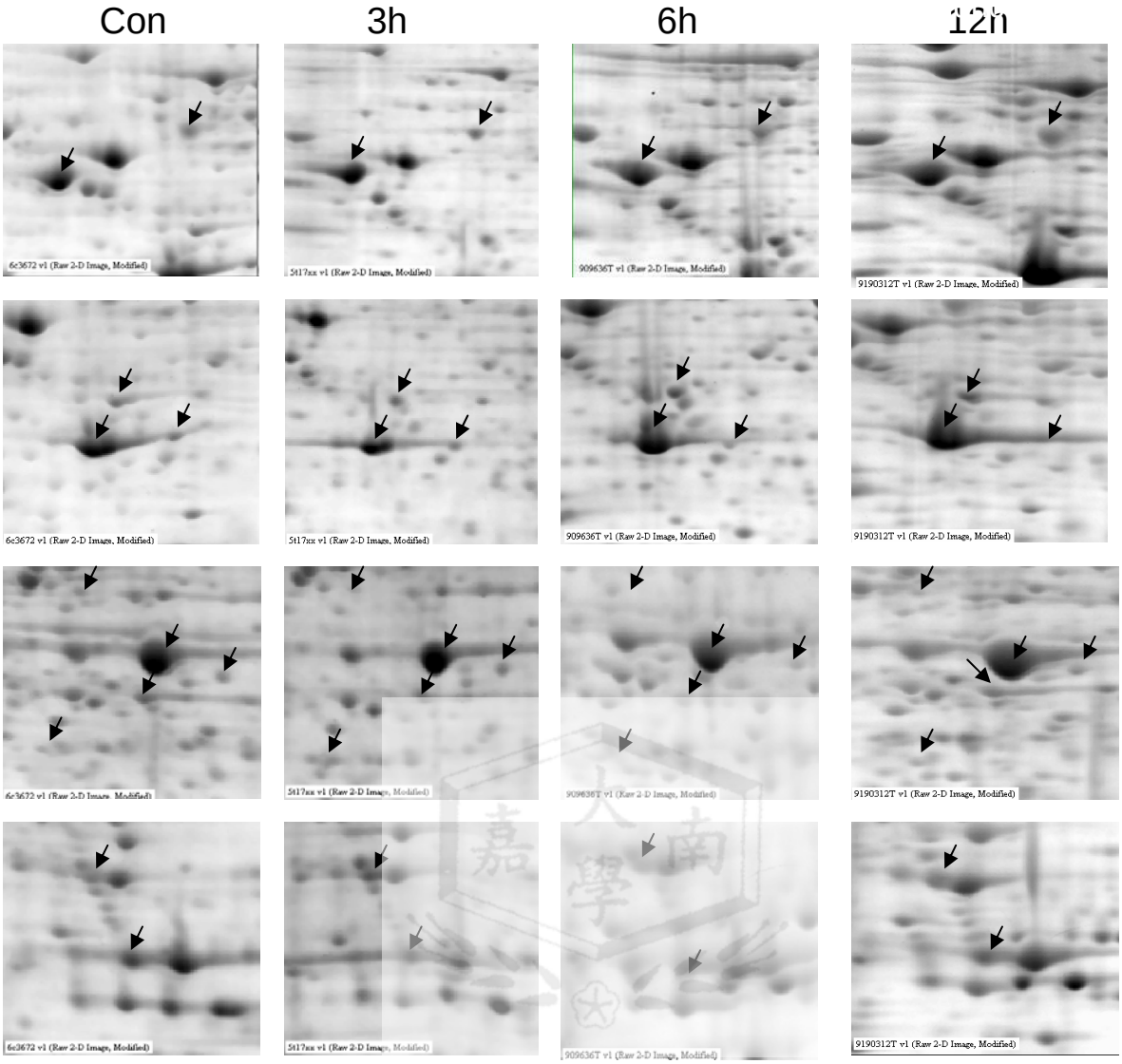


Fig. 5. Close-up views of down-regulated proteins in total cell lysates of KU812 cells when PMA+A23187 treatment at different time periods.

出席國際學術會議心得報告

計畫編號	NSC 95-2313-B-041-011-
計畫名稱	以蛋白體學、RNA 干擾和免疫促進寡核酸技術探討魚類膠原蛋白引起之食品過敏機制
出國人員姓名 服務機關及職稱	呂雅蕙 嘉南藥理科技大學 生活應用與保健系　助理教授
會議時間地點	2007/4/11-4/15 Davos Congress Center Davos, Switzerland
會議名稱	World Immune Regulation Meeting
發表論文題目	Studies on the cross-reactivity of allergens from seafood based on IgE-binding activity

一、參加會議經過

世界免疫調控會議由瑞士過敏和氣喘研究組織(Swiss Institute of Allergy and Asthma Research, SIAF)舉辦，在瑞士沃達斯國際會議中心舉行，會議期間為民國 96 年 4 月 11-15 日。

本次與會人員超過一千人，分別來自於 20 多個國家或地區，我國有三篇論文發表參加，分別來自成大醫院、中研院生化所、嘉南藥理科技大學等。大會共有 63 篇口頭論文、48 篇研習會論文和 277 篇壁報論文。總共由 28 個單元論文發表，包含了專題演講、口頭報告、壁報論文及學術團體競賽等，以及 8 個研習會和 5 個週邊會議。論文展示期間，與瑞士當地學者討論到水產品過敏在亞太地區與瑞士的分佈情形，以及蛋白質體學在過敏原檢測上的應用性。

以下先針對學術上重要新穎的講題摘要整理。講題：Development and maintenance of CD4+CD25+ T regulatory cells，這是大會第一場演講，也直接討論目前最重要的免疫分子 T 調節細胞。轉錄因子 Foxp3 在 T 調節細胞的形成與維持上扮演重要角色。Dr. Alexander Rudensky 先以基因突變方法建立 Foxp3 功能喪失之小鼠和人類模型，發現 T 調節細胞無法產生且從年紀很小的個體發現多重器官遭遇致命性的自體免疫攻擊。IL-2 會影響 Foxp3 的功能性表現。Foxp3 會維持 T 調節細胞不同型態間表現的平衡，而且 T 調節細胞的一致性需要 Foxp3 連續的表現與參與 T 調節細胞的形成。講題：Foxp3 positive Tregs: generation, lifestyle and function，Foxp3 表現型 T 調節

細胞可被胸腺中 T 細胞受體受質誘發產生，而這些受質是由胸腺內皮細胞的 MHC II 分子所表現。另一方面，已活化或記憶性 T 細胞的抗原刺激無法誘發產生 T 調節細胞，但是經由基因工程將 Foxp3 表現於 T 細胞可轉化成 T 調節細胞。Foxp3 需要先跟其他轉錄因子 NFAT 形成複合物後才會調控基因，可能是因為 Foxp3 無法獨立調控基因，需要先與特定基因序列結合，才足以轉換 T 調節細胞。活化 T 調節細胞以備證實會參與免疫反應的各階段程序如活化、增生、分化、形成記憶性細胞、分泌細胞激素和細胞顆粒。因此，T 調節細胞不但可避免而且可逆轉不要的免疫反應，可用以作為免疫疾病預防和治療的展望。T 調節細胞可透過釋放免疫抑制細胞激素如 TGFβ、IL-10 和 adenosine 來達成免疫抑制作用。講題：Transcriptional control of Th1, Th2 and T regulatory cell balance，感染或自體免疫疾病會促進 Th1 或 Th17 細胞生長，而 Th2 細胞是過敏疾病的主因。T 調節細胞可調控 Th1 和 Th2 細胞的擴展，達成免疫耐受性或免疫平衡。轉錄因子藉由 Th1 和 Th2 表現型的極性差異來調控 Th1、Th2 和 T 調節細胞之間的轉型作用。Dr. Carsten Schmidt-Weber 發現作用於 Th2 之細胞激素 IL-4 出現在 T 原型細胞(naïve T cells)轉型初期作用時，會直接與 Foxp3 促進子 GATA3 結合而負向調控 Foxp3。以 IL-4 處裡小鼠也會抑制 T 調節細胞的表現量。因此，在過敏疾病中再誘發免疫耐受作用以達到免疫抑制是有困難的。講題：Epigenetic control of the Foxp3 locus in regulatory T cells，在 Foxp3 locus upstream 的 exon-1 轉錄能力且有很多 CpG motif。這區段中高度 DNA demethylation and hyperacetylation of histones 存在 Foxp3+CD25+CD4+ T 調節細胞。在抑制子細胞型態形成階段亦有染色子重組現象。講題：Mechanism of immune tolerance to allergens，Dr. Mubeccel Akdis 提出過敏的乒乓球理論，原發炎因子如細胞激素和化學激素等在過敏細胞和靜止細胞間的運動以調節過敏發炎現象。並將負向調控氣喘之過敏發炎因子歸類：1)過敏物忽視(allergen ignoreance)，增加支氣管基底膜厚度以在過敏原和免疫分子間形成物理性屏障，例如黏膜分泌的 IgA 和黏液等；2)發炎細胞清潔作用(clearance of inflammatory cells)，3)免疫抑制作用(immune suppression)，例如調節性樹突細胞、T 調節細胞等。

二、與會心得

以上為瑣碎的一般事項，現將一些現象條列整理如下：

1. 歐洲免疫學學會常定期在歐洲境內不同城市舉辦有關免疫學、過敏學、免疫分子機制、免疫治療等主題之相關會議。
2. T 調節細胞(T regulatory cell, Treg)是本次會議最熱門的研究標的，也

累積最大量的研究能量。

3. 此次會議很多學生或年輕學者出席，可見在歐洲免疫學界有年輕化的趨勢。
4. 本次研討會台灣學者參與較少，可能沒接觸到會議通知訊息。
5. 瑞士交通網尤其是鐵路網絡相當密集且運輸頻繁，卻少有誤點情況。雖然交通與生活費用昂貴，但是優良的服務品質值得我們效法。
6. 註冊時發生小插曲，名牌上國家註明 CHINA，經告知主辦單位後，更改為 Taiwan, R.O.C.，但是摘要書上的錯誤標示已無法補救。

綜合此次大會場地、會議流程、動線安排、食宿方面等，深信未來台灣若舉辦此類相關之會議，學術成果絕對是相當可以預期。