行政院國家科學委員會專題研究計畫 成果報告

過敏性氣喘與自體免疫性疾病關連之蛋白體研究與免疫特性分析(第 2 年)
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行政院國家科學委員會補助專題研究計畫 成果報告

過敏性氣喘與自體免疫性疾病關連之蛋白體研究與免疫特性分析

Proteome analysis and immunologic characterization of correlation between allergic asthma and autoimmune disease

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中文摘要

自體免疫與過敏疾病皆為重要的免疫調節異常所導致的疾病，但傳統上認為兩者的致病機制並不相同。近來有越多的文獻發現自體免疫疾病與過敏疾病之間，存在著許多新的關聯性。臨床證據也顯示部分過敏病人會轉變為紅斑性狼瘡病人，亦或是狼瘡病人會併發過敏症狀等。然而，自體免疫與過敏在致病機轉的關連性仍有許多未知之處。因此，我們利用同時患有過敏性氣喘與紅斑性狼瘡病人(SEL with allergic asthma, SLE+AA)、異位性皮膚炎病人(atopic dermatitis, AD)以及非過敏性病人(non-atopic, NA)的周邊血液單核細胞 (peripheral blood mononuclesar cells, PBMCs)，以 EBV 病毒進行轉型作用後，建立穩定且可以長期培養的轉型 B 淋巴球細胞株為細胞實驗模式與系統。接著，利用第二群塵蟎過敏原 (美洲室塵蟎過敏原：Der f 2, DF2) 刺激轉型 B 淋巴球細胞株，透過蛋白質體學、免疫學與細胞生物學的分析方法，分析 B 淋巴球之蛋白質表現變異、免疫特性的改變以及細胞功能的變化。

我們的研究發現，以 DF2 刺激對塵蟎過敏之紅斑性狼瘡病人之轉型 B 淋巴球，會導致轉型 B 淋巴球的去聚集化現象，並進而抑制其細胞增生。將不同的轉型 B 淋巴球進行二維電泳分析比較，發現 DF2 會誘導轉型 B 淋巴球 (SLE+AA 與 AD) 產生蛋白質表現之改變，但是對非過敏性病人 (NC) 之 B 淋巴球則無顯著之影響。這些具有顯著差異之蛋白質經膠內消化，質譜分析鑑定結果為 Uncharacterized protein C17orf59、Myozenin-3、Profilin、Alpha-Enolase 以及 Glutathione S-transferase。其中 profilin 已知在過敏病人中會有表現上升之現象，而 Alpha-Enolase 與 Glutathione S-transferase 則是被報導為自體抗原之一。

綜合以上結果，我們發現在經由 DF2 刺激之後，狼瘡病人同時患有過敏性氣喘者(SLE+AA)的 ET-B 細胞之自體抗原 (alpha-enolase, glutathione-S-transferase) 的表現量會下降，至於是否還有更多的自體抗原的蛋白質表現有下降的情形，則需更進一步地探討。因此，未來我們會利用其他方式再去證實與討論此一這個結果，包括利用各種不同的病人血清進行免疫分析與 LC-MS/MS 等，更深入地分析與比較經 DF2 進行刺激之後所造成的蛋白體變化，以及其免疫生理意義。

關鍵字：自體免疫、B 淋巴球、塵蟎過敏原、二維膠體電泳、免疫分析，質譜分析

Abstract

Both autoimmune and allergic diseases are resulting from deregulation of immune responses, but their pathological mechanisms are traditionally recognized as diverse reactions. Interestingly, mounting evidences show that some unclear relationship presents between autoimmune and allergy, e.g. SLE patients simultaneously suffering from allergic asthma and *vice versa*. However, the mechanisms lying in the relationship between autoimmune and allergy remain unclear. For this reason, the present study aim to investigate the mechanisms by using a combination of proteomic and immunologic methods. Blood samples from SLE patients, SLE with allergic asthma patients, atopic dermatitis patients and non-atopic subjects were collected. The immortalized B lymphocytes were obtained by using isolated peripheral blood mononuclear cells (PBMCs) with EB virus transformation and were cultured in RPMI-1640 medium. With stimulation of DF2, a major house dust mite allergen, the changes of cell morphology and cellular protein expression were analyzed.

Our results revealed that DF2 treatment significantly reduced the clustering of EBV-transformed B lymphocyte (ET-B) from SLE+AA, which was a remarkable property of immortalization by EBV transformation. Interestingly, DF2 treatment showed little influence on the clustering of ET-B from AA and NC. These ET-B cells, with or without DF2 treatment, were further lyzed and analyzed by two-dimensional gel electrophoresis. Several proteins with differential expression among the SLE+AA, AA and NC were identified by using peptide-mass fingerprinting; they were uncharacterized protein C17orf59, myozenin-3, profiling, alpha-Enolase and glutathione S-transferase (GST). Among the identified proteins, profiling is reported that its expression is raised in allergic patients. Additionally, alpha-enolase and GST are both the well-known autoantigens. Further immunoblots showed that SLE+AA serum contained IgG against crude proteins from A549 cell line, an human epithelial cell line, but AA and NC did not the autoreactive IgG.. Moreover, ELISA assays revealed that DF2 treatment significantly induced production of IgE from ET-B cells but showed little effect on production of IgG.

In conclusion, we established a stable ET-B cell model to investigate the correlation between SLE and allergic asthma. According to the results, we suggested that DF2 allergen which triggered allergic responses also suppressed the clustering of ET-B cells, induced IgE production of ET-B cells and led to a different protein expression profiles. Moreover, the identified DF2-induced proteins including two well-known autoantigens revealed that allergen treatment may also alleviate the expression of autoantigens.

Keywords: Autoimmune, B lymphocyte, house dust mite, 2-D gel electrophoresis, immunoassay, proteomic analysis.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune multi-organ disease characterized by the production of autoantibodies in serum directed against a wide spectrum of nuclear, cytoplasmic, and cell membrane autoantigens (1). Although the etiopathogenesis of SLE remains unclear, Epstein-Barr virus (EBV) has been implicated in the pathogenesis of SLE (2-4). However, these previous studies failed to detect a consistent association and significance in the pathogenesis of SLE (5,6). EBV is a ubiquitous human gamma-herpesvirus with cell growth transforming ability that predominantly infects B-lymphocytes and elicits strong immune responses in the infected host (7). The data of previous studies suggest that immune responses in SLE arise as a consequence of antigenic cross-reactivity of anti-EBV antibodies (8-10). A recent study showed that autoantigenic epitope from some SLE patients positive for antibodies to Ro (60 kDa) directly cross-reacts with a peptide from the latent viral protein EBV nuclear antigen-1 (EBNA-1) (8). Additionally, other two studies have also demonstrated that immunization with the peptide of PPPGMRPP, derived from the amino acid sequence of Sm B/B', or with the closely related sequence PPPGRRP of EBNA-1, could generate anti-Sm humoral autoimmunity and induce SLE in rabbits, suggesting such antibodies could contribute to disease (9,10). Accordingly, the association of EBV infection and SLE was supported by the evidences which IgG anti-EBV viral capsid antigen (VCA) in serum and EBV DNA in peripheral blood mononuclear cells (PBMC) were detectable in 116/117 (99%) and 32/32 (100%) young SLE patients.

Allergic inflammation is observed in human subjects and experimental animals in response to characteristic pathogens that include helminths and fungi and after exposure to certain shed or secreted products that include pollen and a wide variety of insect, animal, and mite antigens. Although T and B cells are clearly required for allergic lung inflammation, innate signaling molecules, especially the C3a anaphylatoxin (11,12), are also essential (13). Of additional importance to the control of allergic inflammation is the signal transducer and activator of transcription 6 (STAT6) signaling pathway, which is critical for the development

of T helper (T_H2) cells, eosinophils, and IgE-secreting B cells and is an important mediator of experimental allergic lung disease that resembles allergic asthma (14,15). STAT6 is further required for the homing of T_H2 cells to the lung after allergen challenge by transcriptionally regulating production of allergy-specific chemokines, such as CCL17 and CCL11 (16). Although pathogen-associated molecular patterns such as endotoxin have been linked to allergic lung inflammation under some experimental conditions, neither they nor allergenic pathogens induce STAT6 directly; indeed, no signaling pathway activated by allergenic organisms has been specifically linked to allergic inflammation and disease (17). Consequently, the innate response to allergenic organisms that might be important for the control of allergic lung disease remains unknown.

Inhalation allergy to house dust mite (HDM) is among the most prevalent allergic diseases worldwide (18,19). Allergy to HDMs causes symptoms typical for type-I immediate hyperreactivity, such as rhinoconjunctivitis and asthma. The allergic phenotype is characterized by a persistent inflammation in the airway mucosa and the presence of allergen-specific IgE. Allergen specific IgE binds to high-affinity receptors on the surface of mast cells and basophiles, thereby enabling these cells to become activated by allergen. After activation, mast cells and basophiles release mediators, such as histamine, leukotrienes, enzymes, etc., that are the direct causes of the allergic symptoms. The binding of allergen molecules to receptor-bound IgE and the cross-linking of these on the surface of mast cells and basophiles is therefore the key event in the triggering of a cascade of events leading directly to allergic symptoms. A thorough understanding of the molecular events leading to activation of mast cells and basophiles is thus facilitated by the elucidation of the three-dimensional structure of the allergen molecule. Furthermore, allergen structures facilitate the rational design of allergen variants with reduced IgE binding, which has possible uses in specific allergy vaccination (20).

Several species of house dust mites have been identified contributing to the environmental exposure of human beings (21). The most prevalent species belong to the genus

Dermatophagoides (i.e., *D. pteronyssinus* and *D. farinae*). Because the HDMs are taxonomically related, the different species contain homologous allergens with structural similarities, which cause IgE cross-reactivity (22). Patients sensitized to one species therefore often also react to the other species (23,24). Several proteins derived from house dust mites have been characterized as allergens. The most important allergens in terms of prevalence of reactivity are the group 1, 2, 3, and 9 allergens, to which >90% of mite allergic patients have IgE (25). Group 1 and group 2, however, account for most of the IgE on a quantitative basis. Whereas the biological function of the group 2 mite allergen has not yet been identified, the other mentioned allergens are proteases. Groups 3 and 9 are serine proteases, and group 1 is a cysteine protease located in the alimentary canal of the mite (22).

In the present study, we established three stable EBV-transformed B (ET-B) cell lines (SLE+AA, AA and NC) and used a major HDM allergen, Der f 2 (DF2) to treat the ET-B cell lines to investigate the changes of proteomic and immunologic characterizations. Our results revealed that DF2 significantly influenced the protein expression and the antibodies (Abs) production of SLE+AA, but not of AA and NC ET-B cells. Additionally, the DF2 treatment alleviated the expression of alpha-enolase and glutathione S-transferase, both the well-known autoantigens, the finding which suggested that DF2 treatment may ameliorate the autoreactivity triggered by autoantibodies in SLE+AA patients.

2. Materials and Methods

2.1 Cell lines

All the blood samples were obtained from SLE and allergic patients as part of their diagnostic program and the Institutional Review Board (Veterans General Hospital-Taichung) approved all the procedures. B lymphocytes were obtained from patients with SLE, allergic asthma and both. Briefly, whole blood samples were collected using EDTA-coated tubes and then peripheral blood mononuclear cells (PBMCs) were separated from blood using Ficoll-plaque gradient centrifugation. The isolated PBMCs

were transfected with EBV and the transformed B lymphocytes were collected and cultured for the following experiments. Transformed B lymphocyte was culture in RPMI-1640 medium supplemented with 10% FBS, sodium pyruvate, sodium glutamate and antibiotics.

Human epithelial cell line A549 was a gift from Dr. Tzang, Institute of Biochemistry and Biotechnology, Chung Shan Medical University. A549 was cultured in DMEM medium supplemented with 10% FBS and antibiotics.

2.2 Chemicals

Materials and reagents for 2-DE analysis were purchased from Bio-Rad Laboratory Inc., they included IPG strips (pH 3-10, 7-cm and 17-cm), Bio-Lite (2%) ampholyte and mineral oil. Tris base, CHAPS, urea, thiourea, Coomassie Brilliant blue R-250, MTT (3-[4,5-dimethylthiazol-2-yi]-2,5-diphenyl-tetrazolium bromide), sodium chloride, sodium phosphate, potassium hydrogen phosphate, PMSF, aprotinin, leupeptinin, sodium pyrophosphate, sodium fluoride, sliver nitrate and sodium bicarbonate were purchased from Sigma-Aldrich Inc. Sequence-grade trypsin was purchase from Promega Inc. Methanol, acetic acid, TCA, TFA, acetone, acetonitrile, ethanol, formic aicd, formaldehyde and 2-propanol were purchased from J.T. Baker Inc.

2.3 Cell viability assay

Cell viability was analyzed by using the MTT assay (26) with modification (27). Briefly, each well was treated with 50 μL of MTT reagent, incubated at 37°C for 60 min, mixed with 550 μL of MTT solubilizing solution, and incubated at 37°C for 10 min. The reduction of MTT to a colored formazan was assessed by measuring the absorbance of the sample on a spectrophotometer at 570 nm. Absorbance values were converted to number of viable cells by reference to a MTT standard curve constructed for each cell line under investigation.

2.4 Fluorescent microscope

The morphology of cells with different treatments was monitored by light microscope (200x). Fluorescent microscope was performed by using mouse anti-DF2 Abs and rabbit anti-mouse IgG Ab conjugated with FITC. Briefly, treated cells were collected and washed by PBS. The washed cells were reacted with mouse anti-DF2 Abs (1/500 dilution) for 1 h at room temperature. After wash by PBS, rabbit anti-mouse IgG Ab conjugated with FITC was added and reacted for 1 h at room temperature in dark. After wash by PBS, the reacted cells were monitored by fluorescent microscope and the photography was acquired (200x).

2.5 Immunoblotting

After gel electrophoresis, the proteins were transferred to a PVDF membrane (Immobilon-P, Sigma; St. Louis, MO, USA) for 1 h at 300-mA constant current in a semidry transfer chamber (TE77, Hoefer Scientific; San Francisco, CA, USA). The blotted membranes were blocked with 5% (w/v) skimmed milk, and then incubated for 2 h at room temperature with a 1/2000 dilution of goat anti-human IgG antibody conjugated with peroxidase (Abcam Ltd, UK) or rabbit anti-human IgE antibodies (Abs)(Abcam). The bound Abs were detected using 1/3000 dilution of HRP-conjugated goat anti-rabbit IgG Abs (PerkinElmer, Shelton, CT) and ECL chemiluminescence substrate (Millipore).

2.6 ELISA assay

After treated with DF2, the ET-B cells were span down and the culture medium was collected for analysis of IgE and IgG production. Briefly, 100 μL of culture medium was loaded into a 96-well ELISA microplate (Millipore) and incubated for 16 h at 4^{o}C. After the incubation, medium was removed and the microplate was washed by TBST and then blocked by 5% (w/v) skimmed milk. 100 μL of primary antibodies (rabbit anti-human IgE, 1/2000 dilution or goat anti-human IgG conjugated with HRP, 3/1000 dilution)(Abcam) was added into the blocked microplate and incubated for 2 h at room temperature. After the incubation, the microplate was washed with TBST and then incubated with 100 μL

goat anti-rabbit IgG conjugated with HRP (1/3000 dilution)(PerkinElmer) for 2 h at room temperature. After wash with TBST, the color development was performed by using TMB (Sigma) and the absorbance at 450 nm was measured.

2.7 Cell lysis and protein extraction

Cultured cells were collected by centrifugation (800g x 5 min) and washed by ice-cold PBS twice. The collected cells were resuspended in lysis buffer (50 mM Tris-HCl, pH 7.2; 1 mM PMSF, NaF and NaPPi, 10 μg/mL aprotinin and leupeptin) and stood on ice for 30 min. Homogenization was performed by Dounce homogenizer. After centrifugation (20,000g x 15 min), the supernatant regarded as crude extract was transferred into a new eppendorf and stored at -70^{o}C. Protein concentration of the samples was determined by a modified Bradford's assay (18) using a microplate reader (SpectraMAX 360 pc, Molecular Devices, Sunnyvale, CA) at 595 nm and BSA as standard.

2.8 SDS-PAGE and two-dimensional gel electrophoresis

Proteins in SF (30 μg per lane) were electrophoresed on 12.5% SDS-polyacrylamide gels and stained with Coomassie billinant blue R-250 (CBR)(Sigma). For 2-DE, 500 μg of SF or 300 μg of AR-SF were prepared in 2-D rehydration buffer (8 M urea, 4% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3-10; Bio-Rad, Richmond, CA) and loaded through active rehydration method (50V for 16h). The first dimension of 2-DE was run on IPG strips (ReadyStrip^{TM} IPG Strip, pH 3-10, 17 cm for SF and 7 cm for AR-SF; Bio-Rad) on a horizontal electrophoresis system (PROTEAN^{TM} IEF Cell, Bio-Rad). The 17 cm-IPG strips for SF were focused in a six-step procedure, first ramped to 300 V for 1 min, linearly increased to 1000 V, 2000 V, 4000 V and 8000 V over 1 h, 1 h, 2 h and 6 h respectively, finally maintained at 8000 V until 55000 Vh in total focusing at 20^{o}C.

2.9 Gel image documentation and analysis

All developed gels were scanned at 300 dpi resolutions in TiFF format by using an optical flatbed scanner with transparency unit (Xlite, Avegene, Taipei, Taiwan). 2-DE gel images were analyzed by using Phoretix 2D Elite software (Nonlinear, Durham, NC). The

detection stringencies were as follows. Phoretix 2D Elite (operator size 25): sensitivity 9700, noise factor 3, background 2.

2.10 In-gel digestion and peptide extraction

The CBR-stained protein spots on 2-D gel with differential level between OA and control subjects were excised, and then destained with 1 mL 50% ACN in 25 mM NH4HCO3 and dried in a SpeedVac concentrator as described previously (21). Protein was digested for 16 h at 37oC with sequencing-grade trypsin (0.1 mg/mL; Promega Madison, WI) in 25 mM ammonium bicarbonate. The resulting peptides were extracted twice with 50 μL 1% TFA and 0.1% TFA/50% ACN respectively. The collected extracts were lyophilized, resuspended in 10 μL 0.1% TFA and desalted on ZipTip C18-microcolumn (Millipore, Bedford, MA). Elution was vacuum dried and resuspended in 0.5% TFA/30% ACN. Protein identification was carried out by LC-MS/MS analysis.

2.11 Protein identification.

Peptide extracts were mixed with CHCA (5 mg/mL) directly onto the MALDI target (1 L of the solution was applied to a plated sample holder and introduced into the mass spectrometer after drying). Peptide masses were obtained by using a Bruker Biflex IV MADLI-TOF-MS (Bruker Daltonics, Billerica, MA). The accelerating voltage was 20 kV. For unbiased collection of MS signals, each spot was randomly ionized by laser with 25% of the maximal intensity for 40 trials, in which encompassed 200 laser shots. For PMF, all collected MS signals were integrated into a summary spectrum and processed by using FlexAnalysis and Biotools software (Brukers Daltonics). The processed data was analyzed by using the MASCOT searching engine (www.matrixscience.com). The search parameters were defined as follows: Database, NCBInr; Taxonomy, Homo sapiens; enzyme, trypsin; fixed modification, carbamidomethylation; peptide MS tolerance, 0.2 Da; and allowance of one missed cleavage.

MS/MS analysis was performed on an integrated nanoflow LC/MS/MS system (Thermo finnigan, San Jose, CA) comprising a Finnigan Surveyor HPLC system with an

autosampler, a peptide trap column (Michrom BioResources, Auburn, CA) plus reversed-phase capillary column (PicoFritTM; 5 μm BioBasic® C18 [Thermo Electron], 300 Å pore size; 75 μm x 10 cm; tip 15 μm, New Objective, Woburn, MA), and a LCQ DECA XP plus mass spectrometer (Thermo finnigan) equipped with a nanospray ionization source, operated under BioWorkTM 3.1 control. The digested peptides were eluted from the trap and separated on the reversed-phase capillary column with a 30-min linear gradient of 0-60% acetonitrile in 0.1% formic acid/water at a flow rate of approximately 0.1 μL/min after split. The instrument was operated in data dependent mode fragmenting (relative collision energy, 35%; isolation width, 2.0 Da; dynamic exclusion) on the four most abundant ions in each survey scan. The processed data was analyzed by using the MASCOT searching engine (www.matrixscience.com). The search parameters were defined as follows: Database, NCBInr; Taxonomy, Homo sapiens; enzyme, trypsin; fixed modification, carbamidomethylation; peptide MS tolerance, 0.8 Da; MS/MS tolerance, 0.2 Da, and allowance of one missed cleavage.

2.12 Statistical analysis.

Statistical analysis was performed using the SigmaStat version 3.0 for Windows (SPSS Inc. Chicago, IL). The results are presented as mean ± standard deviation (SD). The statistical significance between groups was determined using Student's t test. A p value less than 0.05 was considered statistically significant.

3. Results

3.1 Effects of DF2 allergen on SLE+AA and AA ET-B cell lines

Three stable ET-B cells were established by using PBMC from SLE+AA, AA and NC patients and EB-virus transformation. As shown in Figure 1A, DF2 treatment significantly reduced the number of clusters for ET-B cells. Because the clustering is one of important properties of ET-B cells, which facilitates cell proliferation, it is suggested that DF2 may suppress the proliferation or the viability of ET-B cells of SLE+AA. Further investigation was

performed by using MMT assay. As shown in Figure 1B, after 6-day treatment, DF2 significantly suppressed cell proliferation in ET-B cells of SLE+AA, but it showed no significant effects on ET-B cells of AA patients. The results provided evidences that DF2 suppressed the cell viability of ET-B cells of SLE+AA, but not of AA. Additionally, the proliferation of ET-B cells of SLE+AA (control) greater than of AA may indicate that ET-B cells of SLE+AA were more activated than of AA.

3.2 Effects of DF2 allergen on Ab production from SLE+AA B lymphocyte

The immunologic property was assayed by detection of Ab production. As shown in Figure 2A, the IgE production from ET-B cells of SLE+AA and AA reached to maximum after 4 days and 8 days of DF2 treatment, respectively. The increase of IgE production from ET-B cells of AA indicated that the ET-B cells remained immunologic characterization of B cells from allergic patients. Comparing to ET-B cells of AA, the IgE production from SLE+AA reached to maximum after 4 days of DF2 treatment. The duration to reach maximum of SLE+AA was shorter than of AA. The differences of IgE production profiles suggested that ET-B cells of SLE+AA showed more sensitivity to DF2 than of AA.

Interestingly, production of IgG from ET-B cells of SLE+AA and AA was little influenced by DF2 treatment. As shown in Figure 2B, the change of IgG production from ET-B cells was not significant in both SLE+AA and AA. The results were consistent with general responses of B cells, either atopic or non-atopic, to allergen treatment.

3.2 Effects of DF2 allergen on protein profiles of ET-B cells

DF2 treatment has been demonstrated that affects the cell morphology, cell viability and IgE production of ET-B cells of SLE+AA. To further investigate the influences of DF2 treatment on ET-B cells, 2-D gel electrophoresis (2-DE) and silver staining was performed. As shown in Figure 3, the protein expression of ET-B cell of SLE+AA was significantly altered by DF2 treatment. After quantitation and comparison of the protein spots on 2-D gels, the differentially expressed proteins were excised and in-gel digested, following identified by using MALDI-MS and LC-MS/MS analysis. The significantly down-regulated proteins were

indicated by arrows and identified as list in Table 1. These identified proteins were uncharacterized protein C17orf59, myozenin-3, profiling, alpha-Enolase and glutathione S-transferase. Among the identified proteins, profiling is reported that its expression is raised in allergic patients. Importantly, alpha-enolase and GST are both the well-known autoantigens. The findings suggest that DF2 treatment may suppress the expression of autoreactive proteins leading to ameliorate the severity of SLE.

4. Conclusions

We have established several stable ET-B cell lines, which is a useful tool for investigate the relationship between autoimmune disease SLE and allergic asthma. By using the cell model, we find that DF2, a major allergen of HDM, significantly suppress the cell clustering and increases IgE production. With advance of proteomic approach, several proteins suppressed by DF2 are identified, which includes important autoantigens. Together, we first provide evidences that indicate HDM allergen may alleviate the production of autoantigens.

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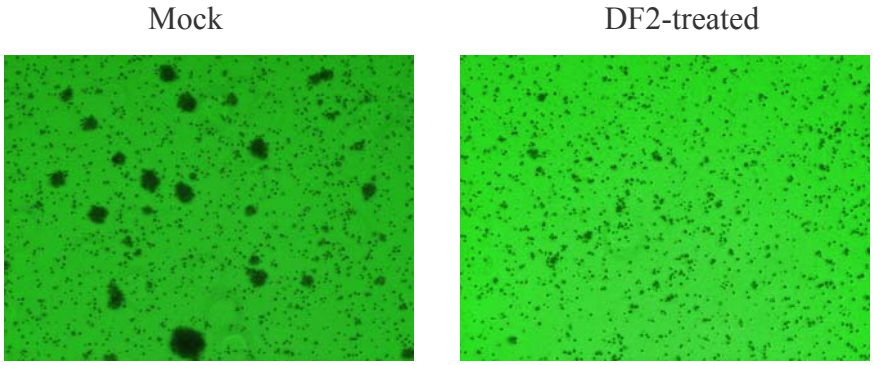
6. Tables and Figures

Table I. Identification for proteins in 2D-PAGE by PMF

Spot	Protein ID	MW (KDa)	pI	match pick no.
1	Uncharacterized protein C17orf59	23.6	5.0	4
2	Myozenin-3	27.2	9.4	6
3	Profilin	15.1	8.4	2
4	Alpha-Enolase	47.2	7.0	5
5	Glutathione S-transferase	25.7	6.9	4

Figure 1. Effects of DF2 allergen on ET-B cells of SLE+AA

A. Change of cell morphology by DF2. Mock, treated with BSA (5μg/mL); DF2-reated, treated with DF2 (5μg/mL). The reaction was performed for 5 h at 37°C.



B. Change of cell viabilty by DF2. SA-B, ET-B cell line from SLE+AA patient; AT-B, ET-B cells from AA patient. DF2 treatment was performed by using DF2 (5μg/mL) in PRMI-1640 without serum supplement.

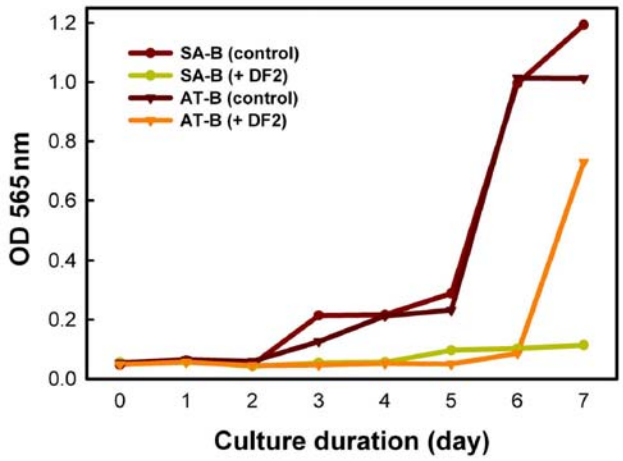
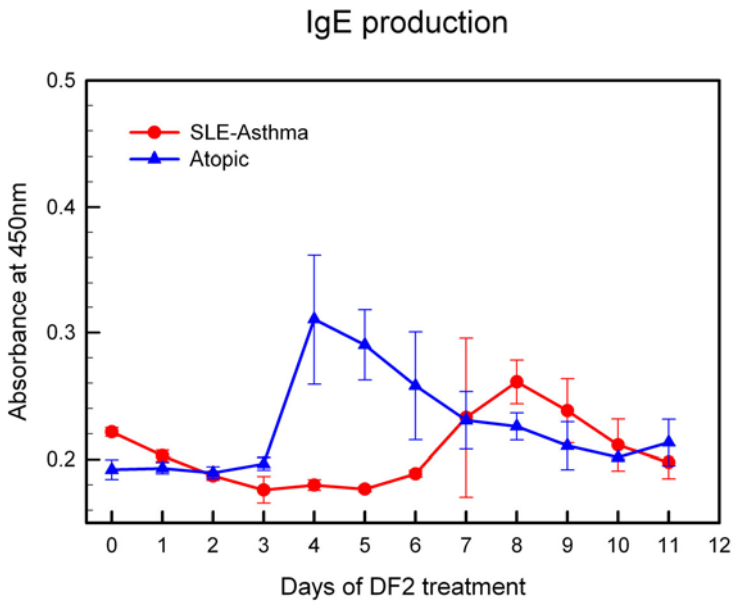


Figure 2. Effects of DF2 on antibody production from ET-B cell lines. SLE-Asthma, from SLE+AA patient; Atopic, from AA patient.

(A) Effects of DF2 on IgE production from ET-B cell lines.



(B) Effects of DF2 on IgG production from ET-B cell lines.

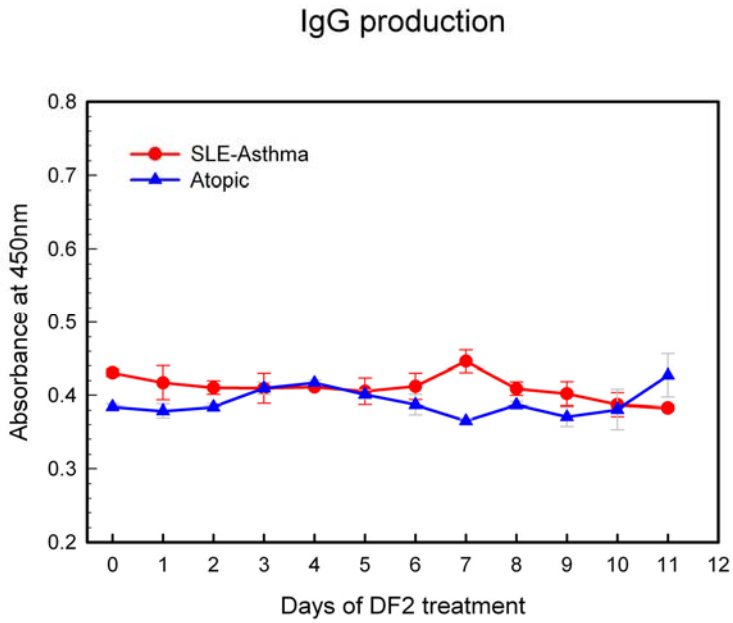


Figure 3. Effects of DF2 treatment on 2-D protein profile for SLE+AA of ET-B cell lines

(A), mock control; (B), treated with 5 μg/mL DF2 for 24h at 37°C. The electrophoresed gels were stained with silver staining.