**Vol.3, No.7, 423-431 (2011)** **Health**
doi:10.4236/health.2011.37070

# T cell responses to der f 2 mite allergens in thai allergic patients

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Received 3 May 2011; revised 31 May 2011; accepted 27 June 2011.

## ABSTRACT

***Dermatophagoides farinae* group 2 (Der f2) is a highly polymorphic allergen that shows a distinct pattern of sequence divergence. The effect of the variants on antibody and T cell responses has not been compared. The aim of the present study was to evaluate IgE binding, transcription and translations for IL-5, IFN-$\gamma$ and TGF-$\beta$ induced by mite allergens. Sera from 24 HDM-allergic patients and 20 non-allergic subjects were measured for IgE reactivity by ELISA. PBMC was cultured with mite allergens (Df, rDerf2) and mitogen (PHA). The supernatants and cell pellet obtained were evaluated for cytokine production by ELISA and cytokine gene expression by RT-PCR, respectively. Four patients showed IgE reactivity to both allergens. Five patients showed IgE reactivity to Df. Other allergic patients and all non-allergic subjects did not show IgE reactivity to mite allergens. Both allergens showed similar levels of IL-5 and IFN-$\gamma$ transcriptions in allergic patients and non-allergic subjects. The rDer f2 induced IL-5 protein from allergic patients higher than non- allergic subjects, while Df showed IL-5 protein from allergic patients similar to non-allergic subjects. Df induced IFN-$\gamma$ protein from allergic patients higher than non-allergic subjects whereas rDer f2 induced IFN-$\gamma$ protein from allergic patients similar to non-allergic subjects. The ratio of IFN-$\gamma$ to IL-5 production after stimulation with rDer f2 was higher in non-allergic subjects than in allergic patients. Our data demonstrated that the changes in the sequence of rDer f2 compared with native Df had effect on cytokine production in both allergic patients and non-allergic subjects.**

**Keywords:** Dermatophagoides Farinae; IgE; IL-5; IFN-$\gamma$; TGF-$\beta$

## 1. INTRODUCTION

*Dermatophagoides farinae* is a species of house dust mites (HDM) which belong to the family Pyroglyphidae. The mite has been ubiquitously found in common houses in Tropical and subtropical areas [1]. *D. farinae* has been recognized as important source of allergens associated with allergic diseases such as asthma, rhinitis, and atopic dermatitis [2]. Group 2 allergen (Der f2 from *D. farinae*) is products of single gene, but it shows frequent allelic variation affecting several amino acids. Recombinant Der f2 (rDer f2) is a convenient molecule for investigat- ing polymorphic proteins. It can be readily produced as a highly allergenic recombinant protein which has been used for X-ray crystallography and other conformational analyses [3]. Seven amino acid substitution differences from native Df are S57N, L58I, D59N, I63V, F75Y, V76I, and I88A. Different variants of Der f2 have dif- ferent immunoglobulin E binding activities [4]. The amino acid substitutions can render T cell epitopes ac- tive or inactive. The cytokine pattern of T cell responses induced by different variants of rDer f2 was also found to differ even with a single amino acid substitution [5].

Allergen specific T cell producing Th1 and Th2 cytokines can be detected in blood of atopic adults [6,7]. The cause of this allergen-specific Th2 predominance in atopic could be related to a dysfunction of the regulatory cytokine that modulate Th1 and Th2 responses. Experimental and theoretical data support the idea that activation of Th2 cells leads to production of IL-4, IL-5 and IL-13 that are cytokines involved in the synthesis of specific IgE and eosinophylic inflammation [8]. On the

 

other hand, Th1 cells, that produce IFN-γ, inhibit the biological effects of Th2 cytokine and are involved on delay type hypersensitivity [9]. In spite of this observation and of their mutual inhibitory properties, the Th1/Th2 differentiation does not explain some features such as increasing IFN-$\gamma$ production by PBMC stimulated by Der f antigens in atopic children [10] and the presence of activated Th1 cells in asthmatic patients with bronchial inflammation and hyper-responsiveness [11] and in some experimental models of asthma [12]. TGF-$\beta$ inhibits T cell differentiation and proliferation of both Th1 and Th2 cells and attracts macrophages, dendritic cells and other inflammatory cells to sites of antigen exposure. TGF-$\beta$ inhibits the function of these cells once they are activated [13]. The increased levels of regulatory cytokines TGF-$\beta$ in atopic patients suggest attempts to control the inflammatory response [14].

The major diagnosis methods of allergic diseases include skin pick tests (SPT) and serum specific IgE detection. Since the SPT possesses simple, quick, inexpensive and highly sensitive characteristics, it has long been one of most essential methods for diagnosis of type I allergic diseases in spite of the rapid development of science and technology over the past century [15,16]. SPT allows the detection if IgE-mediated sensitivity with simple device and low risk of causing an allergic reaction [15,16]. Enzyme linked immunosorbent assays (ELISA) is accurate and precise measurement of IgE and cytokine production. Reverse transcriptase-polymerase chain reaction (RT-PCR) allows detection of cytokine gene expression due to it has good reproducibility and specificity [17].

The aims of the present study were to evaluate humoral and cellular immune responses to *D. farinae* in allergic patients and non-allergic subjects by determineing the levels of specific IgE to Df and rDer f2, the cytokine (IL-5, IFN-$\gamma$ and TGF-$\beta$) gene expression and production by PBMC stimulated with both allergens.

## 2. METERIALS AND METHODS

### 2.1. Mite Extracts

Total HDM extracts of *D. farinae* (Df) was obtained from mite cultures provided by Siriraj Dust Mite Center of Services and research, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Briefly, mite culture was stirred in phosphate buffered saline (PBS), pH 7.4 for overnight at 4 degree Celsius (˚C). The mixture was centrifuged for 20 min at 12,000 xg at 4˚C. The supernatant was filtered through No.1 Whatman paper and through 0.22 μ filter. The sterile mite extract was then stored frozen at –20˚C [18].

### 2.2. Recombinant Allergen Group 2 of *Dermato-phagoides Farinae* (rDer f 2)

rDer f2 was produced by donated from Assistant Professor Surapon Piboonpocanun from the Institute of Molecular Biosciences, Mahidol University, Thailand. The positive transformants containing the integration of interested genes were selected for small-scaled expression. Briefly, positive transformants (rDer f 2) and plasmic vecter (pPICZ) of *Pichia pastoris* (*P. pastoris*) [19] were grown in yeast extract peptone dextrose (YPD) containing 100 μg/mL Zeocin™ at 30˚C with vigorous shaking for approximately 2 days. The starter cultures were added into 5 mL of fresh buffered minimal glycerol complex medium (BMGY) until the absorbance of BMGY was 0.1 at optical density (OD) 600 nanometer (nm). After that, the starter culture with OD 0.1 was cultured until the absorbance of BMGY was 5.0. The BMGY cultures were centrifuged at 10,000xg for 5 min, and the cell pellets were resuspended in buffered minimal methanol complex medium (BMMY) using 1/5 volume of the original culture volume (approximately 1 mL). To induce expression of recombinant allergens, absolute methanol was added to a final concentration of 3% v/v to the BMMY culture every 24 h. The culture media were collected after 3 day induction. The collected culture media were centrifuged at 12,000 xg in a bench-top micro centrifuge for 3 min at room temperature. The supernatant was dialyzed.

The proteins were separated by SDS-PAGE followed by transferring the fractionated proteins to a nitrocellulose membrane using method as described by the manufacturer with a wet blotting apparatus (Bio-Rad, USA). The blotted membrane was blocked overnight at 4˚C. The membrane was further incubated with monoclonal anti-group 2 antibodies (mAb 1D8 clone, Indoor biotechnologies Ltd, Manchester, UK). The membrane was incubated with Biotinylated rabbit-anti mouse IgG solution (1:5000) (SouthernBiotech, Birmingham, USA) and BCIP/NBT 1 component substrate (KPL, USA).

### 2.3. Subject

A total of 24 patients with perennial allergic rhinitis and a positive Skin prick test (SPT) to house dust mite allergen (Der p and Der f), were selected for this investtigation by Dr. Tharit Muninnobpamas of Department of Otolaryngology, Pramongkutklao Hospital, Bangkok, Thailand. As the control group, 20 healthy subjects with no history of allergic diseases and a negative SPT to all aeroallergen extracts tested were included. The study donor was approved by the Ethics committee in Human Research of the Phramongkutklao Hospital and written informed consent was obtained from all volunteers.

 

### 2.4. Skin Prick Test and Serum Sample

The commercial extract of Der p (*Dermatophagoides pteronyssinus*) and Der f (*D. farinae*), CR (American Cockroach), Grass (Bermuda grass pollen), Dog (Dog Epithelium), Cat (Cat pelt), Weed, Smut (*Sporobolus indicus*), M (*Penicillium notatum*, *Aspergillus niger*, Drechslera, *Cladosprium spharospermum*, Alternaria), manufactured by allertech, Australia. Histamine dihydrochloride (10 mg/mL) was used as positive control and normal saline (0.9% w/v) was used as negative control. A mean wheal 3 mm in diameter larger than the negative control (diluents) was considered to be positive. In parallel, blood samples (5 mL) were collected from all individuals and the serum was stored at –20˚C.

### 2.5. Measurement of Specific IgE

IgE reactivity to a panal of proteins from Df and rDer f2 was determined by ELISA [5]. Briefly, dilute each allergen in coating buffer (50 nM carbonate/bicarbonate buffer) at concentration of 1000 ng/mL Two-hundred µl of each allergen was coated in each well of 96-well poly polystyrene microtiter wells (Nunc™, Denmark) and incubated at 4˚C overnight. The blocking buffer was added into each well and incubated at 37˚C for 60 min. The sera at dilution of 1: 10 was added into the well and incubated at 37˚C for 120 min. biotinylated-labeled mouse anti-human IgE antibody (SouthernBiotech, Birmingham, USA) at dil 1: 1000 was added into each well and incubated at 37˚C for 60 min. During each step, the plates were washed with PBs-Tween20 for 5 times. The plates was added with streptavidin-peroxidase solution at dil 1: 1000 and incubated at 37˚C for 60 min. The color reaction was developed by adding ABTS® peroxidase substrate (KPL, USA). Absorbance of IgE antibody was measurced at OD405nm with a Wallac 1420 microplate reader (Auto DELFIA Wallac 1420; Wallac, Turku, Finland).

### 2.6. Cell Culture

PBMC dissolved in AIM-V medium seeded into 12-well plates at $1 \times 10^6$ cells/mL per well were stimulated with 100 µL allergen extracts (30 µg) (Df and rDer f), mitogens (PHA), or medium alone and then incubated at 37˚C in 5% $CO_2$. The cells were harvested using centrifugation at 250 × g for 15 min 4˚C at 1, 3, 5 and 7 days post-incubation. The supernatants and the cell pellets were frozen at –80˚C until required.

### 2.7. Enzyme-Linked Immunosorbent Assay (ELISA)

The concentration of IL-5, IFN-$\gamma$ and TGF-$\beta$ in the supernatant were measured with ELISA Ready-SET-Go kits (eBioscience, Minneapolis, MN) according to manufacturer instructions using an ELISA reader (Auto DELFIA Wallac 1420; Wallac, Turku, Finland). All assays were performed in two independent experiments. The concentration was calculated using a linear-regression equation obtained from the standard absorbance values.

### 2.8. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

The total RNA was extracted from the cell pellets using TRI Reagent (Molecular research center, USA) according with the manufacturer's instructions. The total RNA (1 µg) was incubated at 45˚C for 60 min using the first-strand cDNA synthesis kit (Intron Biotechnology, Korea) to prepare the cDNA. The primer sequences were as follows: **IL-5,** 5'-GAG GAT GCT TCT GCA TTT GAG TTT-3' and 5'-GTC AAT GTA TTT CTT TAT TAA GGA CAA-3'; **IFN-$\gamma$,** 5'-AGC TCT GCA TCG TTT GGG TTC-3' and 5'-GTT GGC CCC TGA GAT AAA GCC-3'; **TGF-$\beta$,** 5'-ACC ACT GCC GCA CAA CTC CGG TGA C-3' and 5'-ATC TAT GAC AAG TTC AAG CAG AGT A-3'; **$\beta$-Actin,** 5'-ATC TGG CAC ACT TCT ACA-3' and 5'-GTT TCG TGG ATG CCA CAG GAC-3'. PCR was carried out with an initial denaturation at 95 for 5 min for 5 min, then 35 cycles of denaturation at 95 for 30 sec, annealing at 55 for 30 sec, extension at 72 for 30 sec and final exten- sion at 72 for 10 min. $\beta$-Actin was used as an internal control for each PCR reaction. The final PCR products were separated on 1% agarose gels and then visualized by ethidium bromide staining.

### 2.9. Statistical Analysis

Data were expressed as the means (±SEM). The statistical differences were analyzed using the Mann-Whitney U-test in the SPSS statistical software package (Version 13 Chicago, IL, USA). A significant value was defined as $p < 0.05$.

## 3. RESULTS

### 3.1. Subject Characteristics

The demographic and clinical characteristics of the study subjects (**Table 1**) were distributed into two groups according to SPT positivity to commercial HDM-total extract: 1) HDM+ group: patients with positive SPT to HDM-total; 2) HDM- group: Non-allergic subjects with negative SPT to HDM-total and aeroallergens. Rhinitis was the most frequent clinical diagnosis observed in HDM+ group but not be observed in HDM-group. The

 

**Table 1. Demographic and clinical characteristics of the study subject.**

| Characteristics | Groups | |
|---|---|---|
| | HDM+ | HDM- |
| Number of subject (N) | 24 | 20 |
| Age (years, means ± SD) | 35.17 ± 12.06 | 30.75 ± 5.46 |
| Gender (male/female) | 10/14 | 9/11 |
| Clinical diagnosis (N, %) | 24, 100% | 20, 100% |
| Categorized by allergens (N, %) | | |
| Der f, Der p, | 9, 37.50% | 0 |
| Der f, Der p, CR, Dust, | 6, 25.00% | 0 |
| Der f, Der p, CR, Dust, Smut | 2, 8.33% | 0 |
| Der f, Der p, CR | 2, 8.33% | 0 |
| Der f, Der p, CR, Weed | 1, 4.17% | 0 |
| Der f, Der p, Cat, Dog | 1, 4.17% | 0 |
| Der f, Der p, CR, Smut | 1, 4.17% | 0 |
| Dust, Der f, Der p, CR, Dog | 1, 4.17% | 0 |
| Dust, Der f, Der p, CR, Weed, Cat, Dog | 1, 4.17% | 0 |

HDM+; HDM-allergic subjects, HDM-; Non-allergic subjects, Dust; House dust, Der f; *Dermatophagoides farina*, Der p; *Dermatophagoides pteronyssinus*, CR; American Cockroach, Smut; *Sporobolus indicus*, Dog; Dog epithelium, Cat; Cat pelt.

HDM+ group showed the mean wheal size to HDM-total and histamine dihydrochloride (positive control) were ≥ 3 mm. In contrast, all subjects did not show the mean wheal size to normal saline which was used as negative control. HDM-group showed a negative SPT to mite extracts as well as other aeroallergens tested according to the selection criteria used.

The highest SPT was observing at 37.5% from patients suffering from two HDM-totals (Der f and Der p) whereas the least SPT was observing at 4.17% from patients suffering from two HDM-total and other aeroal lergens.

### 3.2. T Cell Responses to Df and rDer f2 Allergens at Molecular Level

IL-5, IFN-$\gamma$, TGF-$\beta$ cytokine genes from allergic patients and control subjects were stimulated with PHA, Df, rDer f2, pPICZ and Medium alone by RT-PCR. The results revealed that PHA, Df, rDer f2, pPICZ and Medium alone induced IL-5,and IFN-$\gamma$ genes in both allergic patients and control subjects. In contrast, PHA, Df, rDer f2 pPICZ and Medium alone did not induce TGF-$\beta$ gene in either allergic patients or control subjects (**Figure 1**). It was suggesting that IL-5, IFN-$\gamma$, TGF-$\beta$ genes from allergic patients were similar to those from non-allergic subjects at molecular level.

### 3.3. T Cell Responses to Df and rDer F2 Allergens at Cellular Level

IL-5, IFN-$\gamma$, TGF-$\beta$ cytokine protein production from allergic patients and control subjects were stimulated with PHA, Df and rDer f2 by ELISA. For allergic patients, PHA, Df and rDer f2 induced IL-5 production at 217.76 ± 27.84, 7.47 ± 0.61 and 58.33 ± 4.92 pg/mL, respectively. For non-allergic subjects, PHA, Df and rDer f2 induced IL-5 production at 165.50 ± 16.61, 4.43 ± 0.174 and 6.31 ± 0.85 pg/mL, respectively (**Table 2**). Correlation between PHA and rDer f2 were found in allergic patients ($r = 0.253$; $p < 0.05$) but not found in non-allergic subjects (**Figure 2**). No correlation between PHA and Df were found in allergic patients and non-allergic subjects (data not shown). For allergic pa-

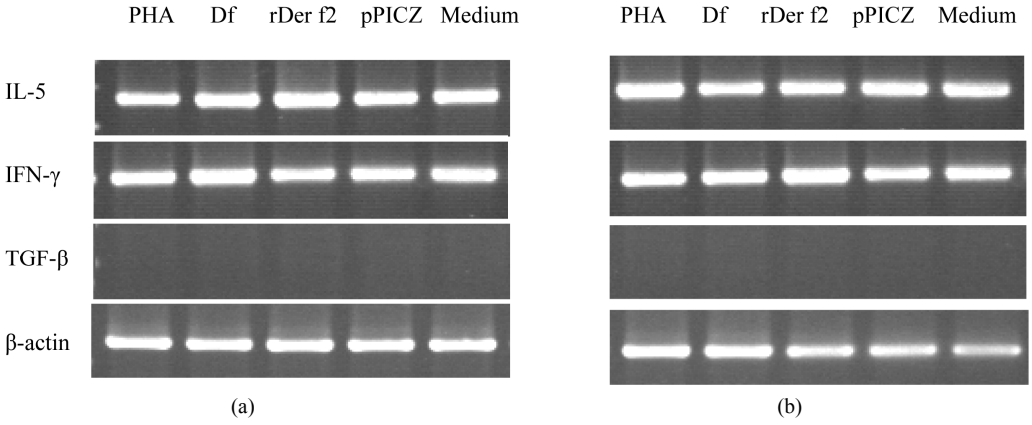


**Figure 1. IL-5, IFN-$\gamma$ and TGF-$\beta$ gene expression to PHA, Df, rDer f2, pPICZ and medium compared with $\beta$-actin house keeping gene in PBMC of allergic patients (a) and control subjects (b). (a) Allergic patients, (b) Control subjects.**

 

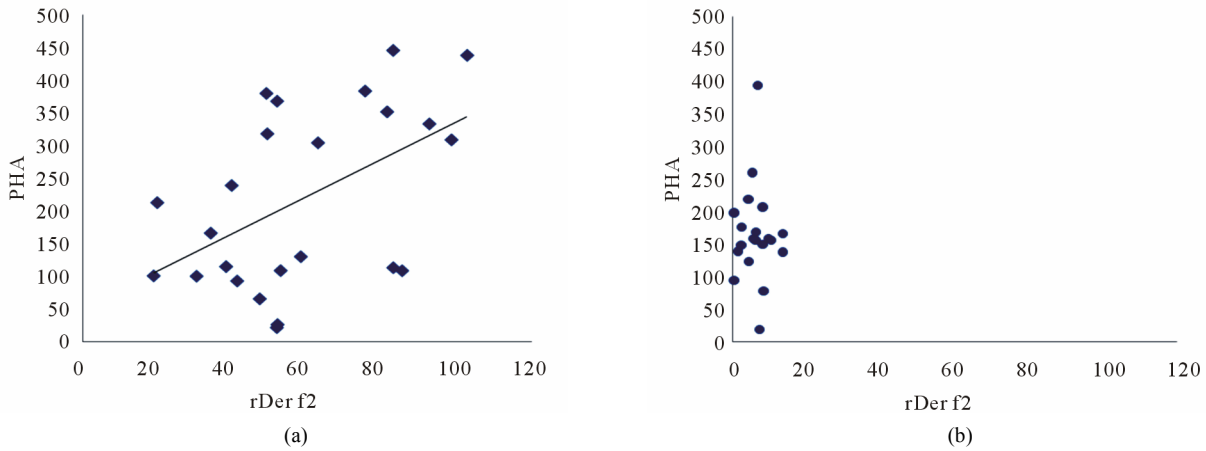


**Figure 2 Correlation of IL-5 production of PBMCs from HDM-allergic patients (a) and Non-allergic subjects (b) to PHA and rDer f2 ($r = 0.253$; $p < 0.05$). (a) HDM-allergic patients, (b) Non-allergic subjects.**

tients, PHA, Df and rDer f2 induced IFN-$\gamma$ production at 863.98 ± 67.78, 70.32 ± 6.74 and 619.62 ± 61.43 pg/mL, respectively. For non-allergic subjects, PHA, Df and rDer f2 induced IFN-$\gamma$ production at 1142.61 ± 78.40, 6.16 ± 0.45 and 410.73 ± 48.56 pg/mL, respectively. No correlation between PHA and Df as well as PHA and rDer f2 was found in allergic patients and non-allergic subjects (data not shown). For allergic patients and non-allergic subjects, PHA, Df and rDer f2 showed level of TGF-$\beta$ from PBMC of allergic patients and non-allergic subjects at <60 pg/m (**Table 2**).

To analyze the effect on Th1/Th2 bias the responses the allergic patients and non-allergic subjects, the data showed that the cytokine release to PHA did not differ from allergic patients and non-allergic subjects. The Df allergen showed IFN-$\gamma$/IL-5 ratio from allergic patients (7.16 ± 0.70) higher than non-allergic subjects (1.43 ± 0.1), suggesting that Df allergen slightly induced Th1/Th2 bias in allergic patients, but not good enough for non-allergic patients. The reverse rDer f2 allergen showed IFN-$\gamma$/IL-5 ratio from non-allergic subjects (53.57 ± 9.71) higher than allergic patients (11.25 ± 1.08), suggesting that rDer f2 induced Th1/Th2 bias in non-allergic subjects better than allergic patients.

## 3.4. Specific IgE Reactivity to Df and rDer f2 Allergens

Level of specific IgE from allergic patients and non-allergic subjects to Df and rDer f2 were measured by ELISA. As shown in **Table 3**, of 24 allergic patients who had been SPT positivity to commercial HDM-total extract, only 4 patients (No. 3, 5, 6 and 7) showed specific IgE to both Df and rDer f2. Only 5 patients (No. 2, 11, 14, 15 and 16) showed IgE binding to Df whereas no patient showed specific IgE to rDer f2. Fifteen patients showed specific IgE values below 0.35 $kU_A/L$ to Df and rDer f2. It was suggesting that the SPT positivity to commercial HDM-total extract was not related to IgE-binding reactivity to HDM allergens in allergic patients. Of 20 non-allergic subjects, all subjects showed specific IgE values below 0.35 $kU_A/L$ to Df and rDer f2 (data not shown). It was suggesting that the SPT negatively to commercial HDM-total extract was related to specific IgE reactivity to HDM allergens in non-allergic subjects. No correlation between the level of IL-5 production and the degree of IgE-binding to the rDer f2 from HDM-allergic patients and non-allergic subjects were observed (**Figure 3**).

**Table 2. Cytokine protein responses to the PHA and house dust mite allergens from HDM-allergic and non-allergic subjects*.**

| | IL-5 (pg/mL) | | IFN-$\gamma$ (pg/mL) | | TGF-$\beta$ (pg/mL) | | IFN-$\gamma$/IL-5 ratio | |
|---|---|---|---|---|---|---|---|---|
| | Allergic | Non-allergic | Allergic | Non-allergic | Allergic | Non-allergic | Allergic | Non-allergic |
| PHA | 217.76 ± 27.84 | 165.50 ± 16.61 | 863.98 ± 67.78 | 1142.61 ± 78.40 | <60.00 | <60.00 | 6.83 ± 1.49 | 10.06 ± 2.98 |
| Df | 7.47 ± 0.61$^{\ddagger}$ | 4.43 ± 0.174 | 70.32 ± 6.74$^{\dagger\ddagger}$ | 6.16 ± 0.45$^{\ddagger}$ | <60.00 | <60.00 | 7.16 ± 0.70$^{\dagger}$ | 1.43 ± 0.12$^{\ddagger}$ |
| rDer f2 | 58.33 ± 4.92$^{\dagger}$ | 6.31 ± 0.85 | 619.62 ± 61.43 | 410.7 ± 48.56 | <60.00 | <60.00 | 11.25 ± 1.08$^{\dagger}$ | 53.57 ± 9.71 |

*Results are presented as means (±SEM), † Significant differences between Allergic Non-allergic groups. ‡ Significant differences between Df and rDer f2 allergens. Cut off of IL-5= 4 pg/mL, IFN-$\gamma$ = 4 pg/mL and TGF-$\beta$= 60 pg/mL.

 

**Table 3. IgE-binding to HDM allergens in HDM-allergic patients.**

| No. | SPT | Allergic history | IgE –binding ($kU_A/L$) | |
|---|---|---|---|---|
| | | | Df | rDer f2 |
| 1. | + | Dust, Der f, Der p, CR, Smut | 11.24 | 3.42 |
| 2. | + | Der f, Der p | 11.07 | 0.40 |
| 3. | + | Dust, Der f, Der p, CR, | 6.09 | 3.40 |
| 4. | + | Dust, Der f, Der p, CR, Dog | 5.27 | 3.18 |
| 5. | + | Der f, Der p, CR, Smut | 5.74 | <0.35 |
| 6. | + | Der f, Der p, Cat, Dog | 5.09 | <0.35 |
| 7. | + | Dust, Der f, Der p, CR, | 3.39 | <0.35 |
| 8. | + | Der f, Der p, CR, Weed | 1.88 | <0.35 |
| 9. | + | Dust, Der f, Der p, CR, | 0.99 | <0.35 |
| 10. | + | Der f, Der p, CR, Weed | <0.35 | <0.35 |
| 11. | + | Dust, Der f, Der p, CR, Smut | <0.35 | <0.35 |
| 12. | + | Dust, Der f, Der p, CR, | <0.35 | <0.35 |
| 13. | + | Dust, Der f, Der p, CR, | <0.35 | <0.35 |
| 14. | + | Dust, Der f, Der p, CR, | <0.35 | <0.35 |
| 15. | + | Der f, Der p, CR, | <0.35 | <0.35 |
| 16. | + | Der f, Der p, CR, | <0.35 | <0.35 |
| 17. | + | Der f, Der p | <0.35 | <0.35 |
| 18. | + | Der f, Der p | <0.35 | <0.35 |
| 19. | + | Der f, Der p | <0.35 | <0.35 |
| 20. | + | Der f, Der p | <0.35 | <0.35 |
| 21. | + | Der f, Der p | <0.35 | <0.35 |
| 22. | + | Der f, Der p | <0.35 | <0.35 |
| 23. | + | Der f, Der p | <0.35 | <0.35 |
| 24. | + | Der f, Der p | <0.35 | <0.35 |

*SPT, skin prick tests; +, SPT wheal response diameter was more than 3 mm; *Dust; House dust, Der f; *Dermatophagoides farina*, Der p; *Dermatophagoides pteronyssinus*, CR; American Cockroach, Smut; *Sporobolus indicus*, Dog; Dog epithelium, Cat; Cat pelt, *Cut off of IgE –binding; 0.35 $kU_A/L$.

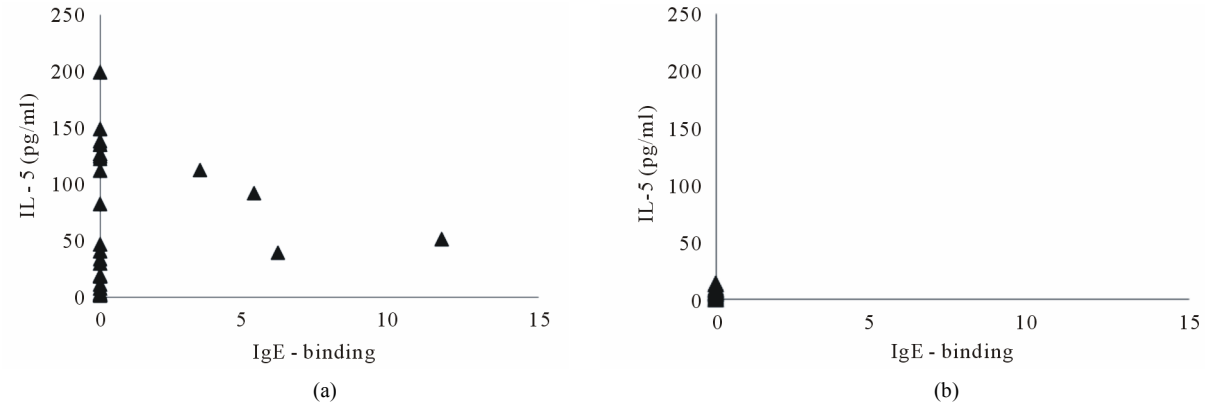


**Figure 3 No correlation (r = 0.01) between the level of IL-5 production and the degree of IgE-binding to the rDer f2 from HDM-allergic patients (a) and Non-allergic subjects (b) were observed. (a) HDM-allergic patients; (b) Non-allergic subjects.**

 

## 4. DISCUSSIONS

House dust mites (HDMs) as a source of allergens and their contribution to allergic diseases, particularly asthma and rhinitis, have been recognized for many years [20]. Allergic rhinitis is an inflammatory reaction of the nasal mucosa, in consequence of an IgE mediated hypersensitive reaction to inhaling allergens, involving different mediators and cytokine cells [21]. Positive skin tests and/or serum IgE antibodies to HDM have been widely demonstrated in genetically predisposed individuals. In the present study, we found only 37.5 % allergic patients presenting positive SPT to both mites (Df and Dp), while 62.5 % of allergic patients sensitized to a panel of aeroallergen. These results were similar to those of our previous studies carried out in another group of allergic patients from Police hospital in Bangkok [2], confirming that the high rate of this concurrent sensitization has certainly complicated the evaluation of the role of *D. farinae and D. pteronyssinus* in these patients, particularly in tropical and subtropical regions where both mites coexist. Owing to glycosylated antigens have been shown to play a crucial role in different pathologies, particularly in fungal infections and allergic diseases [20]. In this context, Df-total extract was fractioned by SDS-PAGE and confirmed by Western blot analysis using 1D8 monoclonal antibody. The recombinant Der f2 allergen derived from *P. pastoris* was also fractioned by SDS-PAGE and confirmed by Western blot analysis. The recombinant Der f2 allergens presented predominantly molecular weight at 14 kDa. Both allergens were used to detect IgE antibody in allergic patients and healthy subjects. We found levels of specific IgE antibody detected by Df extract were higher than those by recombinant Der f2 allergen in allergic patients. These results are consistent with report of Almedida *et al*. [22] and Pipatchaipaisan, [2]. It is implied that specific IgE antibody could be related to natural allergen exposure, thus reflecting a normal consequence of immediate hypersensitivity reactions in allergic patients *in vivo* [22]. In our data, we found that IgE are preferentially involved in this cross-reactivity in some allergic patients to both Df extract and recombinant Der f2 allergen. This result indicated that IgE might be recognizing same epitope in the extract allergen and recombinant allergen.

Several reports revealed that both the IgE-dependent early response and antigen-primed Th2 cells are necessary for a persistent atopic inflammation characterized by the accumulation of activated inflammatory cells [23]. Previous studies have highlighted the role of IL-5 in the development of the chronic phase of atopic inflammation and in the expression of allergic diseases [24-26]. The hypothesis of our study was that immunotherapy may induce the functional modification from Th2 to Th1 phenotype in PBMC. To investigate the modifications of cellular immunity in the mechanism of HDM allergens, we evaluated intracellular IL-5, and IFN-$\gamma$ gene expression as well as production from the PBMC of the allergic patients relative to healthy subjects. We did not find significantly difference in IL-5 or IFN-$\gamma$ gene expression from PBMC of allergic patients and healthy control subjects. This data is consistent with other investigators [21]. However, we found that IL-5 production induced by Df extract was slightly positively correlated in both allergic patients and healthy subjects. On the other hand, we found that IL-5 production induced by recombinant Der f2 allergen was significantly increased in allergic patients relative to healthy subject. This data was consistent with the evidence that activated T cells and the Th2 cytokine IL-5 production is increased in lung of atopic asthmatic subjects [24,27,28]. We found no correlation between serum HDM-specific IgE levels and IL-5 production stimulated in either allergic patients or healthy subjects. This is agreement with data from some but not all studies [6,10,29].

Investigations of allergen-simulated release of IFN-$\gamma$ from PBMC have produced mixed results. Some studies found decreased IFN-$\gamma$ production in subjects with atopic diseases relative to healthy control subjects [14,30,31], whereas other studies reported no difference [32,33]. In our study, there was no significant difference in recombinant Der f2-stimulated IFN-$\gamma$ production between allergic patients and control subjects, while we found that degree of Df-total extract-stimulated IFN-$\gamma$ production was higher in allergic patients than in the control subjects. It has been reported that IFN-$\gamma$ production closely correlated with atopic dermatitis in infants [10]. No absolute definition of a Th1- or Th2-type cytokine response was demonstrated. An excess of Th2 cytokines relative to the Th1 cytokine is implicated in the cause of atopy [34,35]. Studies with PBMC have also analyzed allergen-stimulated cytokine production as the amount of IL-5, Th2 cytokine relative to IFN-$\gamma$, Th1 cytokine [30]. We analyzed cytokine production induced by HDM allergens as the ratio of the IL-5 to IFN-$\gamma$ production in each culture that had a positive response to the stimulators and expressed this ratio as a Th2/Th1cytokine index. We found that for allergic patients and control subjects, the Th2/Th1cytokine index was induced by both HDM allergens, although different degree of Th2/Th1cytokine index was observed, suggesting that HDM is the Th2-type stimulators that are consistent with other reports [36]. Additionally, we found that there is a lower frequency of HDM allergen-responsive T cells in healthy control subjects than in allergic patients. Evidence of our data focuses research atten-

 

tion on mechanisms of T-cell recognition of, and activation by, HDM allergens. It is concluded that both allergic patients and normal subjects had a Th2-type cytokine response to HDM allergen; our data does suggest that patients with atopic asthma have an enhanced Th2 cytokine response to HDM allergen relative to healthy control subjects. Therefore, both HDM allergens increased IL-5 production and Th2/Th1cytokine index in patients with allergic rhinitis compared with normal subjects.

## 5. CONCLUSIONS

The major findings of this study are as follows. First, PBMC from allergic patients with allergic rhinitis and healthy control subjects have the Th2-type cytokine response to HDM allergens. Second, allergic patients do have an enhanced Th2-type cytokine response to HDM compared with control subjects. These data indicate that using of both HDM allergens in immunotherapeutic procedures in allergic patients and healthy subjects cannot be recommended.

## 6. ACKNOWLEDGEMENTS

This study was supported by the Thailand Research Funding No. MRG-WII 525S092 and a partial funding from TRF No. MRG--WII505S061. This study was also supported by Grants No. RGP-2552-06 from Faculty of Science, Silpakorn University The authors would like to thank the patients and nurses from Department of Otolaryngology, Pramongkutklao Hospital, Bangkok. We also thank the students from Silpakorn University. Furthermore, we thanks Associate Professor Dr. Darawan Wanachiwanawin, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University for laboratory facility.

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