

Induced apoptosis of TH₂ lymphocytes in asthmatic children treated with *Dermatophagoides pteronyssinus* immunotherapy

Tsai Y-G, Chien J-W, Chen W-L, Shieh J-J, Lin C-Y. Induced apoptosis of TH₂ lymphocytes in asthmatic children treated with *Dermatophagoides pteronyssinus* immunotherapy.

Pediatr Allergy Immunol 2005;16:602–608. © 2005 Blackwell Munksgaard

Allergen-specific immunotherapy (IT) has been effectively used for the treatment of asthma. Allergen specific IT induced immune tolerance with induction of TH₂ cells anergy remain to be clarified. The aim of this study was to evaluate whether the mite allergen *Dermatophagoides pteronyssinus* (Dpt) specific IT serially decreased IL-4+ /CD4+ (TH₂) lymphocytes and induced apoptosis of TH₂ lymphocytes in asthmatic children. Sixty Dpt-sensitive asthmatic children were randomly assigned to a received IT and an untreated group. *Dermatophagoides pteronyssinus* specific IT treated patients were examined at three time points: before IT, after 6 months of an increased dose phase and with maximum tolerated doses after 1 yr. Peripheral blood mononuclear cells (PBMC) were isolated and cultured for 48 h for cellular staining with CD4+, CD45RO cell phenotypes and interleukin (IL)-4 and interferon- γ expression by fluorescence monoclonal antibodies. Apoptosis was measured using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) method. A simultaneous flow cytometric study using the same permeabilized cell was examined to determine whether apoptosis occurred preferentially in TH₂ lymphocytes. The data demonstrated that Dpt specific IT decreased Dpt-specific IgE levels ($p < 0.01$) after 1 yr of treatment. In addition, decreased CD4+ IL-4+ TH₂ cells with increased CD4+ IFN- γ + TH₁ cells were observed at 6 months and 1 yr after IT treatment ($p < 0.05$). At the same time, apoptosis of CD4+ IL-4+ TH₂ lymphocytes in the IT group had increased after 1 yr of treatment when compared with the results before treatment ($p < 0.001$) and after 6 months of treatment ($p = 0.046$). In addition, CD45RO cells apoptosis mainly occurred after 6 months of IT treatment and after 1-year period of IT treatment ($p < 0.05$). All of the data suggested that Dpt specific IT decreased Dpt specific IgE and CD4+ IL-4+ TH₂ lymphocytes with induction apoptosis of CD4+ IL-4+ TH₂ lymphocytes subsets serially.

**Yi-Giien Tsai¹, Jien-Wen Chien¹,
Woan-Ling Chen², Jeng-Jer Shieh²
and Ching-Yuang Lin¹**

¹Department of Pediatrics, Children's Hospital, Changhua Christian Hospital, Institute of Medical Research, Chang Jung Christian University, Changhua, Taiwan, ²Department of Medical Research, Children's Hospital, Changhua Christian Hospital, Institute of Medical Research, Chang Jung Christian University, Changhua, Taiwan

Key words: immunotherapy; apoptosis

Dr Ching-Yuang Lin, Department of Pediatrics, Children's Hospital, Changhua Christian Hospital, 135 Nan Siau Street, Changhua 500, Taiwan
Tel.: 886-4-7238595 Ext: 1901
Fax: 886-4-7285161
E-mail: 100966@cch.org.tw

Accepted 12 May 2005

Abbreviations: Dpt, *Dermatophagoides pteronyssinus*; TUNEL, Deoxyuridine triphosphate nick end-labeling; IT, Immunotherapy; PBMC, Peripheral blood mononuclear cells; PMA, Phorbol-12-myristate-13-acetate.

Allergen-specific immunotherapy (IT) has been effectively used for the treatment of asthma through reducing asthma symptoms and improving bronchial hyper-reactivity (1–3). TH₂ T lymphocytes (CD4+ CD45RO+) that secrete

cytokines such as IL-4, IL-5, and IL-13, are the principal T lymphocytes involved the allergic inflammation of asthma (4–6). A shift in the balance between the lymphocyte responses from TH₂ to TH₁, such as reduction of IL-4 production and enhancement of IFN- γ expression from peripheral blood CD4⁺ T cells have been proposed as the mechanism in atopic patients undergoing IT (7–9).

In allergic asthma, the over-activation of lymphocytes and eosinophils were found to accumulate in the airway inflammation tissues, partly because of their prolonged survival (10). Apoptosis, a specific morphological feature of activation-induced cell death, can maintain T-lymphocytes homeostasis (11). Some evidence suggested allergen induced apoptosis of T lymphocytes in allergen specific IT treated atopic patients (4, 5). Guerra et al. reported that peripheral blood mononuclear cells (PBMC) culture of 48 h with grass allergen induced apoptosis of TH₂ cells from grass IT treated patients (5). Allergen specific IT induced immune tolerance to exogenous allergen with induction of TH₂ cells anergy may explain the operating mechanism of IT.

Dermatophagoides pteronyssinus (Dpt) is the dominant species of house dust mite allergens in Taiwan, especially in asthmatic children (12, 13). The aims of our studies were to examine whether Dpt specific IT decreased the percentages of TH₂ cells and induced apoptosis of TH₂ lymphocytes in Dpt-sensitive asthmatic children at three time points: before specific IT, after the 6-month increased dose phase and after 1 yr of treatment.

Methods

Subjects

Sixty mildly intermittent to moderately persistent asthmatic children with sensitivity to house dust mites (Dpt) demonstrated by IgE specific test greater than third grade (> 3.5 kU/l) using the CAP system (Pharmacia, Uppsala, Sweden) and positive skin prick test (2+ or more) to Dpt were enrolled in this study. Thirty children were randomly selected to receive Dpt specific IT and 30 did not receive Dpt specific IT. The criteria for selective asthmatic children of clinical severity were based on Global Initiative for Asthma (GINA) guidelines. The 30 Dpt specific IT treated patients were followed for 6 months and each received a maximum monthly-tolerated dose after about 1 yr. In addition, 30 patients were not treated and acted as the allergy control

patients. Twenty subjects with normal serum IgE levels, negative for skin prick tests and no history of allergic symptoms were selected as the healthy control subjects. The children's ages ranged from 5 to 17-year old. Patients were instructed to record their daytime and nighttime symptoms of asthma according to a 0–3 grading (0 = absent, 1 = mild, 2 = moderate, and 3 = severe) (8). Pulmonary function (FEV₁) (Sensomedics, USA) was measured before and after 1 yr of treatment with IT. The study was performed with the approval of the institutional review board and informed consent was obtained from the parents of each child.

Cell culture

Peripheral blood mononuclear cells were isolated from 8 ml heparinized venous blood using a differential centrifugation gradient (Ficoll/Hypaque and Pharmacia). PBMC was isolated immediately when all blood samplings were obtained and none of the children had received steroid medications during the month prior to treatment.

A total of 2×10^5 cells per well were cultured for 48 h on 96-well culture plates in RPMI-1640 culture medium supplemented with l-glutamine (2 mmol/l), HEPES (20 mmol/l), sodium pyruvate (91 mmol/l), streptomycin (50 ng/ml), penicillin (100 IU/ml) and 10% bovine fetal serum (Bio-Whittaker, Walkersville, MD, USA). Phorbol-12-myristate-13-acetate (PMA) at 10 μ g/ml plus ionomycin 0.5 μ mol/l were used as cell stimulation and Brefeldin A 2 μ g/ml (Sigma-Aldrich, St Louis, MO, USA), which inhibits intracellular transport mechanisms, was added 4 h before the end of the culture.

Cell apoptosis and cellular staining procedure

Apoptosis of peripheral blood lymphocytes were studied with DNA strand breaks on the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) kit (Mebstain, Immunotect, France). Briefly, 10^6 cells were fixed with 4% paraformaldehyde for 30 min at 4 °C, washed (for 5 min at 1500 g) with phosphated buffer saline (PBS containing 0.2% BSA) and permeabilized for 5 min on ice with 200 μ l 70% ethanol. After washing, cells were decanted and resuspended in 30 μ l of TUNEL mixture solution or in 30 μ l of TUNEL labeled as a negative control. After 60 min of incubation at 37 °C in a humidified atmosphere, cells were washed three times with PBS containing 0.2% BSA buffer and stained with Fluorescein isothiocyanate

Table 1. (a) Baseline patient characteristics and (b) clinical response with symptoms scores, FEV1 and Dpt-specific IgE changes after IT

(a)					
	IT group		Untreated group		Non-atopic group
Number of patients	30		30		20
Mean age (yr)	9.37 ± 3.36		9.83 ± 3.55		9.11 ± 3.18
Gender (M:F)	18:12		20:10		13:7
Skin prick test Dpt (grade)	3.6 ± 0.68		3.15 ± 0.99		—
(b)					
Groups	IT group		Untreated group		
Period	Baseline	After 1 yr	Baseline	After 1 yr	
Dpt-specific IgE (kU/l)	70.80 ± 35.97	52.36* ± 37.84	61.18 ± 38.87	56.32 ± 38.56	
Asthma scores	3.7 ± 0.35	0.9*,** ± 0.21	3.6 ± 0.33	1.88* ± 0.22	
Mean FEV1 (%)	80.4 ± 7.44	91.3* ± 9.10	80.4 ± 12.11	86.7 ± 12.22	

*Mean $p < 0.05$ when compared to baseline.

**Mean $p < 0.05$ when compared between IT and untreated group.

Values are in mean ± s.d

(FITC)-conjugated monoclonal antibody (mAb) for 30 min. For simultaneous detection of apoptosis, cytokine expression, and surface phenotype of a single cell level, anti-IL-4 phycoerythrin (PE) mAb, anti-IFN- γ phycoerythrin (PE) mAb, anti-CD4 (PC5) conjugated mAb and anti-CD45 RO (ECD) mAb or corresponding isotype control mAbs (Coulter-Immunotech, Miami, Florida, USA) were labeled at the same time. After staining, cells were analyzed using a FACSscan flow cytometer (FC500, Beckman Coulter Inc., USA) acquiring 10,000 events.

Statistic analysis

Data analysis was performed using the SPSS software. Data of each group were found to be normal distribution prior to analysis using the One-Sample Kolmogorov–Smirnov Test. Differences between the means before and after the IT groups were analyzed using the paired Student's t -test. Differences of the means compared with each group were analyzed using ANOVA, followed by the Duncan test. The level of $p < 0.05$ was considered to be significant.

Results

Clinical efficacy of Dpt specific IT

All asthmatic children that underwent Dpt specific IT completed the treatment period, and no systemic side effects were reported. There were no significant differences among the baseline characteristics of the untreated group, IT-treated group and non-atopic individuals irrespective of age and gender. There were also no differences

between the untreated and IT-treated asthmatic patients for the Dpt-specific IgE values and skin prick test values at baseline ($p > 0.05$) (Table 1). During the treatment period, Dpt-specific IgE values were all negative (< 0.35 kU/l) in the healthy subjects. *Dermatophagoides pteronyssinus* specific IT significantly decreased Dpt-specific IgE titers with increased pulmonary function (FEV1) after 1 yr of treatment (Table 1b). All asthmatic patients improved asthma scores but significantly reduced asthma scores in post-IT treated group when compared with the control group ($p < 0.05$) (Table 1b).

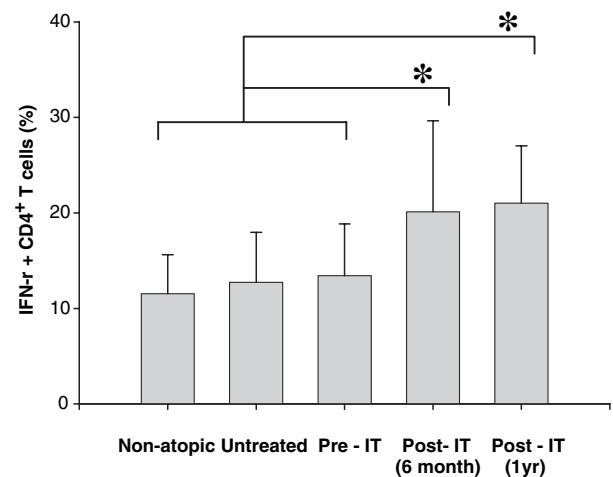


Fig. 1. Percentages of peripheral blood CD4+ T lymphocytes with positive intracellular staining for IFN- γ from non-atopic children, untreated and IT treated asthmatic children on three occasions: before IT, after 6 months and after 1 yr of treatment. Percentages of CD4+ IFN- γ + (TH₁ cells) were significantly increased after 6 months and after 1-year period of IT treatment (*Mean $p < 0.01$).

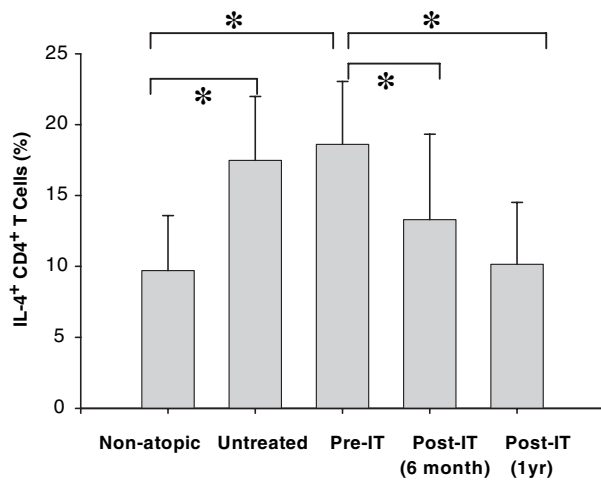


Fig. 2. Percentages of peripheral blood CD4+ T lymphocytes with positive intracellular staining for IL-4 from non-atopic children, untreated and IT treated asthmatic children on three occasions: before IT, after 6 months and after 1 yr of treatment. Percentages of CD4+ IL-4+ (TH₂ cells) were higher in the untreated group and pre-IT group when compared to the non-atopic group. In addition, CD4+ IL-4+ (TH₂ cells) percentages were significantly decreased after 6 months and the 1-year period of IT (*Mean $p < 0.01$).

Dermatophagoides pteronyssinus specific IT increases the IFN- γ proportion and decreases the IL-4+ proportion of CD4+ T cells

In order to analyze the effects of Dpt specific IT on peripheral blood CD4+ T-cell cytokine production, we compared the different percentages of each groups in intracellular IL-4 and IFN- γ staining with CD4+ surface markers. An increased production of IFN- γ by CD4+ T cells was observed after 6 months of IT ($20.11\% \pm 0.10\%$) and after 1 yr of IT treatment ($21.02\% \pm 0.06\%$) when compared with the levels before IT ($13.41\% \pm 0.05\%$) and in the control group ($12.73\% \pm 0.05\%$; $p < 0.05$; Fig. 1). An increase in IL-4 by peripheral blood CD4+ T cells was observed in the untreated group ($17.48\% \pm 4.52\%$) and pre-IT group

($18.61\% \pm 4.44\%$) when compared with the non-atopic group ($9.72\% \pm 3.88\%$; $p < 0.01$). In the IT group, the percentages of CD4+ IL-4+ (TH₂) cells significantly decreased after 6 months

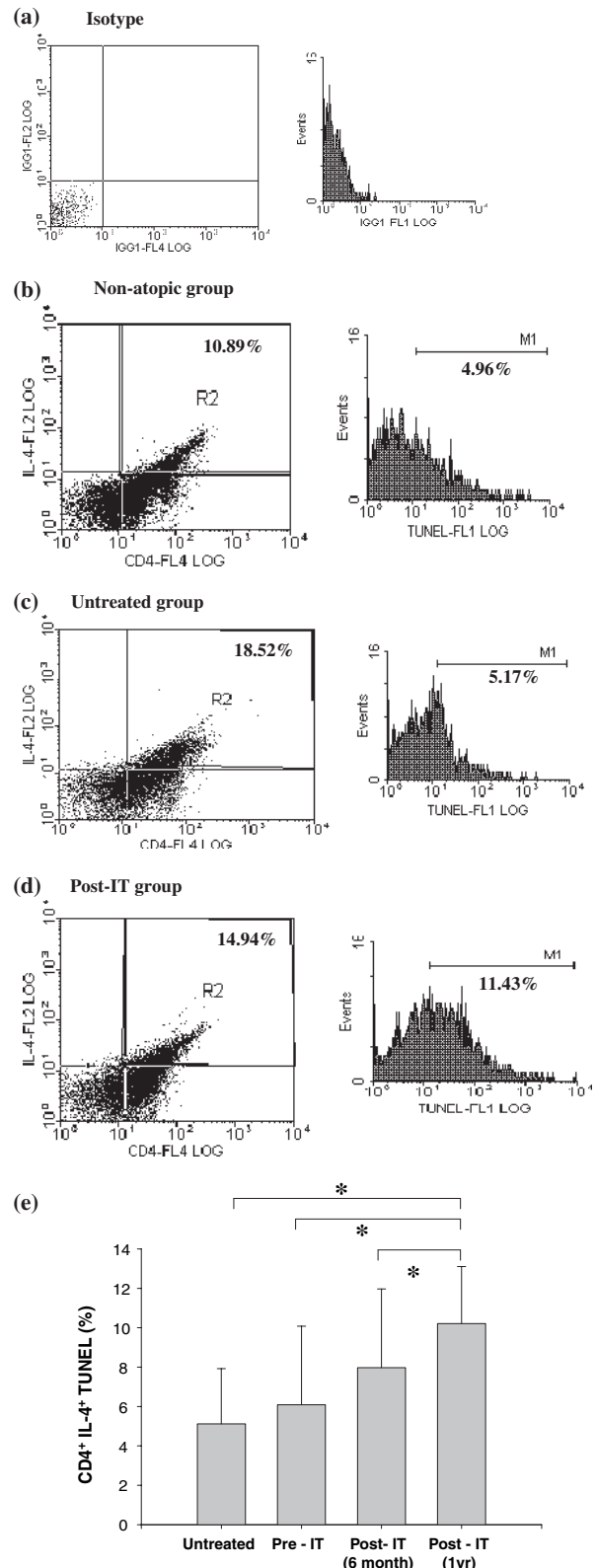


Fig. 3. Study of TH₂ cells apoptosis in different groups as representative examples are as following (a) iso-type control (b) non-atopic group (c) un-treated group (d) post-IT for 1-year period. PBMC were isolated and cultured for 48 h following stimulation with PMA ($10 \mu\text{g/ml}$) plus ionmycin ($0.5 \mu\text{mol/l}$) and Brefeldin A ($2 \mu\text{g/ml}$) lasting 4 h. A simultaneous flow cytometric study after staining with surface marker (anti-CD4 PC5-conjugated mAb), intracytoplasmic cytokine (anti-IL4+ PE conjugated mAb) and TUNEL by using the FITC-conjugated mAb and respective labeled controls. To determine the percentages of TH₂ cells apoptosis, IL-4/CD4+ stained cells were gated and their apoptosis cells expression were determined. (e) Percentages of CD4+ IL-4+ TH₂ apoptosis increased in post-IT after about 1 yr of treatment when compared to the 6 months post-IT and pre-IT treatment (*Mean $p < 0.05$).

of the increased dose phase ($13.30\% \pm 6.02\%$) and 1 yr of the maximum tolerated dose phase ($10.15\% \pm 4.37\%$) when compared with the pre-IT status ($18.61\% \pm 4.44\%$; $p < 0.01$; Fig. 2).

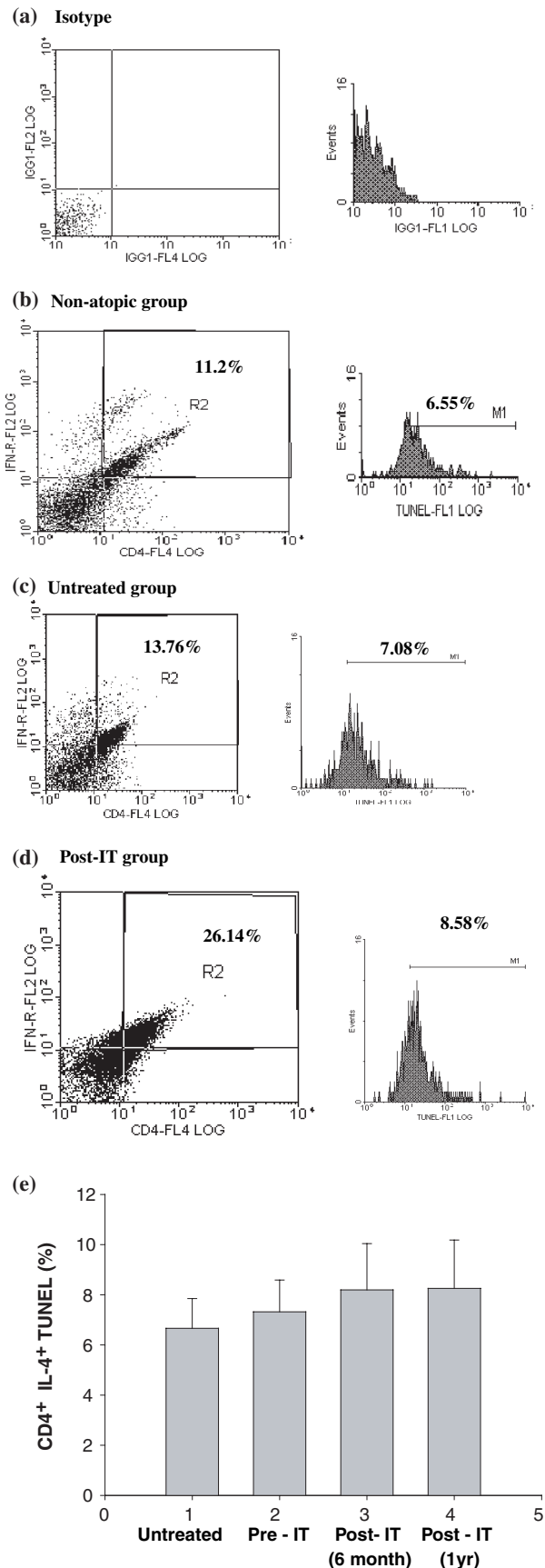
Dermatophagoides pteronyssinus specific IT increased apoptosis on TH₂ lymphocytes

To study whether apoptosis of CD4+IL-4+ TH₂ cells or CD4+IFN- γ + TH₁ cells was induced by Dpt specific IT patients, we employed a simultaneous flow cytometric study in the same permeabilized cell with a surface marker of CD4, intracytoplasmic cytokine with IL-4, IFN- γ , and TUNEL expression. CD4+IL-4+ TH₂ cells apoptosis between the non-treated ($5.12\% \pm 2.80\%$) and pre-IT children ($5.84\% \pm 4.01\%$) were no different ($p > 0.05$). We found a significantly increased percentage of CD4+IL-4+ TH₂ apoptotic cells from Dpt specific IT treated patients that underwent 1 yr of treatment when compared with pre-IT ($10.21\% \pm 2.90\%$ vs. $5.84\% \pm 4.01\%$; $p < 0.001$) and 6 months of treatment ($10.21\% \pm 2.90\%$ vs. $7.97\% \pm 3.67\%$; $p = 0.046$) (Fig. 3). In contrast, no obvious differences in the percentages of TUNEL positive CD4+IFN- γ + TH₁ cells were found after IT ($8.26\% \pm 1.93\%$; 1 yr vs. $7.59\% \pm 1.52\%$; 6 months) when compared with the data from pre-IT ($7.16\% \pm 1.04\%$) and non-treated control patients ($6.66\% \pm 1.19\%$; $p > 0.05$; Fig. 4).

Apoptosis of CD45RO lymphocytes on Dpt specific IT treated children

CD45RO cells are the principal types of T cells involving the production of IL-4 in allergic responses (3, 4). To determine whether apoptosis of CD45RO cells also mainly occurred in Dpt specific IT treated patients; a simultaneous study was performed as previously mentioned. Apoptotic changes of CD45RO were mainly increased in post-IT patients after 6 months ($8.04\% \pm 3.37\%$) and 1 yr of treatment ($10.61\% \pm 5.13\%$) when compared to the pre-IT ($3.57\% \pm 1.96\%$) and the non-atopic group ($4.16\% \pm 1.72\%$; $p < 0.05$; Fig. 5).

Fig. 4. Study of TH₁ cells apoptosis in different groups as representative examples are as follows (a) iso-type control (b) non-atopic group (c) un-treated group (d) post-IT for 1-year period. To determine the percentages of TH₁ cells apoptosis, IFN- γ /CD4+ stained cells were gated and their apoptosis cells expression were determined. (e) Percentages of CD4+ IFN- γ TH₁ apoptosis were no difference between each group (*Mean $p > 0.05$).



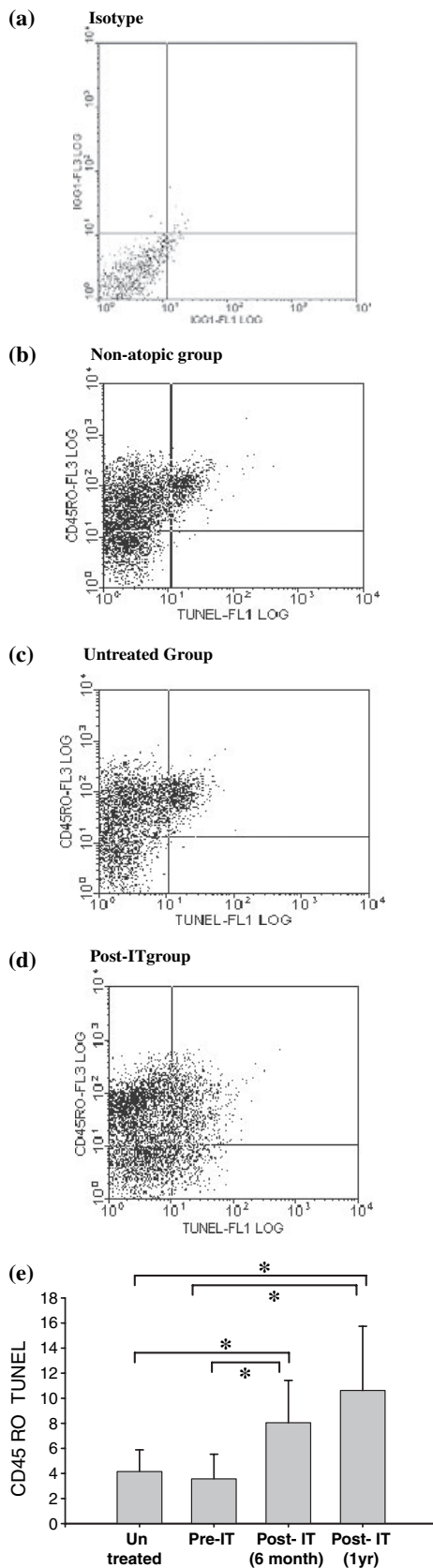


Fig. 5. Apoptosis of CD45RO lymphocytes on representative examples of (a) iso-type control (b) non-atopic group (c) un-treated group (d) post-IT for 1-year period. (e) Specific Dpt treated asthmatic children after 6 months and a 1-year period had higher apoptosis percentages of CD45RO when compared to the non-atopic and pre-IT group (*Mean $p < 0.05$).

Discussion

Allergen specific IT, involving repeated stimulation with the allergen extract, improved symptoms of asthma and modified the cytokine pattern of T-helper cells with decreased IL-4 secretion and enhanced IFN- γ production (7, 9). In the present study, the Dpt specific IT seemed to have the beneficial effects of clinical outcome with decreased asthma scores and increased pulmonary function (FEV1). The mechanism of immune tolerance to exogenous allergen by apoptosis TH₂ T cells may explain the beneficial effects of the specific allergen IT (5, 14). Our results supported that Dpt specific IT caused apoptosis mainly in the TH₂ lymphocytes with IL-4+/CD4+ and CD45RO phenotypes and subsequently decreased the percentages of CD4+IL-4+ TH₂ cells and Dpt specific IgE levels.

Different antigen concentrations influence TH cells repolarization and may play a pivotal role during IT (15). CD4+ T cells isolated from allergic donors produced high levels of IL-4 when stimulated with low concentrations of the allergen, in contrast, little IL-4 production was found when challenged with high concentrations of the allergen (16). Our study revealed that continuously down-regulating IL-4 expression with enhanced IFN- γ production in peripheral blood CD4+ T cells suggests Dpt specific IT modulated the TH₂ type cytokine pattern of CD4+ T cells by accumulation with allergen doses.

Gardner et al. introduced a new concept with the effects of different allergen concentrations on programmed T-cell death (17). PBMC were examined from mite-allergy donors and cultured with different concentrations of mite extract for 14 days. High concentrations of mite allergen culture resulted in the expansion of IFN- γ producing T cells but a decreased in the proportion of CD4+IL-4+ T cells. In addition, the results showed diminished proliferate response accompanied by increased active capase-3 positive CD4+IL-4+ T cells when cultured with highly concentrated mite extract (17). The above evidence indicates T-cell anergy is the potential mechanism of IT, supported by decreased antigen-specific proliferate response, together with a modified cytokine production (16, 17).

In a consistent study, researchers showed that pollen induced TH₂ lymphocytes apoptosis from pollen IT treated atopic patients when cultured with pollen extract for 48 h (5). The researchers proposed that activation-induced cell death of allergen specific TH₂ cells could lead to increased efficacy during IT. We confirmed the above results and more accurately evaluated apoptosis of the Dpt specific TH₂ cells *in vivo* condition during IT without allergen challenge through a serial time course follow up.

Long-term study of allergen-specific T-cell clone from IT treated asthmatic patients suggested TH₂ suppression occurs first and TH₁ stimulation happens later (18). Allergen specific IT reduced allergen skin prick response and serum IgE levels after 1 yr of IT (3, 8). In animal models of IFN- γ CD8⁺ T-cell inhibited allergen-induced eosinophilic inflammation and IgE production (19). Our evidence showed that as down-regulation of IL-4 production of TH₂ cells began at 6 months after the start of IT and Dpt specific IgE antibody levels only decreased after 1 yr of treatment.

IL-10 plays a critical role in the maintenance of self-tolerance by induction of T anergy with blockage of CD28 T-cell co-stimulation (20). In IT treated patients; increased CD4⁺CD25⁺ regulatory T cells with IL-10 production suggested another possible mechanism that modulates TH₂ to TH₁ shift (21, 22).

In conclusion, our results indicated that the shift from TH₂ to TH₁ induced by Dpt-specific IT might be mediated by induction of the programmed cell death process in Dpt-responder CD4⁺IL-4⁺ TH₂ cells in asthmatic children. Although, further studies are warranted to understand how the IL-10⁺CD4⁺CD25⁺ regulatory T cells regulate TH₂ cells anergy and the relationship of immune tolerance to the pathogenesis of asthma during IT.

References

1. ABRAMSON M, PUY R, WEINER J. Allergen immunotherapy for asthma. *Cochrane Database Sys Rev* 2003; 4: CD001186.
2. PHILIP SN. Immunotherapy: 1999–2004. *J All Clin Immunol* 2004; 113: 1013–23.
3. DURHAM DR, TILL SJ. Immunologic changes associated with allergen immunotherapy. *J All Clin Immunol* 1998; 102: 157–64.
4. GUERRA F, CARRACEDO J, MADUENO JA, SANCHEZ-GUIJO P, RAMIREZ R. Allergen induce apoptosis in lymphocytes from atopic patients. *Human Immunol* 1999; 60: 840–7.
5. GUERRA F, CARRACEDO J, SOLANAN-LARA R, SANCHEZ-GUIJO P, RAMIREZ R. TH₂ lymphocytes from atopic patients treated with immunotherapy undergo rapid apoptosis after culture with specific allergens. *J All Clin Immunol* 2001; 107: 647–53.
6. BEYER K, NIGGEMANN B, NASERT S, RENZ H, WAHN U. Severe allergic reactions to foods are predicted by increases of CD4⁺CD45RO⁺ T cells and loss of L-selection expression. *J All Clin Immunol* 1997; 112: 133–9.
7. TILL SJ, JAMES NF, KAYHAN NA, STEPHEN RD. Mechanisms of immunotherapy. *J All Clin Immunol* 2004; 113: 1025–34.
8. MAJORI M, CAMINATI A, CORRADI M, BRIANTI E, SCARPA S, PESCI A. T-cell cytokine pattern at three times points during specific immunotherapy for mite-sensitive asthma. *Clin Exp All* 2000; 30: 341–7.
9. BENHAPONPITAK SI, ORO A, MAGUIRE P, MARINKOVICH V, DEKRUY RH, UMETSU DT. The kinetics of change in cytokine production by CD4⁺ T cells during conventional allergen immunotherapy. *J All Clin Immunol* 1999; 103: 468–75.
10. OHTA K, YAMASHITA N. Apoptosis of eosinophils and lymphocytes in allergic inflammation. *J All Clin Immunol* 1999; 104: 14–21.
11. COHEN JJ, DUKE RC, FADOK VA, SELLINS KS. Apoptosis and programmed cell death in immunity. *Annu Rev Immunol* 1992; 10: 267–93.
12. CHANG YC, HSIEH KH. The study of house dust mites in Taiwan. *Ann All* 1989; 62: 101–6.
13. TSAI YG, LEE MY, YANG KD, CHU DM, YUH YS, HUNG CH. A single dose of nebulized budesonide decreases exhaled nitric oxide in children with acute asthma. *J Pediatr* 2001; 139: 433–7.
14. ROLLAND J, O'HEHIR R. Immunotherapy of allergy: anergy, deletion, and immune deviation. *Curr Opin Immunol* 1998; 10: 640–5.
15. ROGER PR, CROFT M. Peptide dose, affinity, and time of differentiation can contribute to Th1/Th2 cytokine balance. *J Immunol* 1999; 163: 1205–13.
16. SECRIST H, DEKRUY RH, UMETSU DT. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med* 1995; 181: 1081–9.
17. GARDNER LM, O'HEHIR RE, ROLLAND JM. High dose allergen stimulation of T cells from house dust mite-allergic subjects induces expansion of IFN- γ T cells, apoptosis of CD4⁺IL-4⁺ T cells and T cell anergy. *Int Arch All Immunol* 2004; 133: 1–13.
18. ODA N, YAMASHITA N, MINOGUCHI K, et al. Long-term analysis of allergen-specific T cell clones from patients with asthma treated with allergen rush immunotherapy. *Cell Immunol* 1998; 190: 43–50.
19. RENZ H, LACK G, SALOGA J, et al. Inhibition of IgE production and normalization of airways responsiveness by sensitized CD8 T cells in a mouse model of allergen-induced sensitization. *J Immunol* 1994; 152: 351–60.
20. AKDIS CA, JOSS A, AKDIS M, FAITH A, BLASER K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J* 2000; 14: 1666–8.
21. JUTEL M, AKDIS M, BUDAK F, et al. IL-10 and TGF- β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33: 1205–14.
22. FRANCIS JN, TILL SJ, DURPHAN SR. Induction of IL-10 CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J All Clin Immunol* 2003; 111: 1255–61.