附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※

家塵滿過敏原 Der p2 在塵滿過敏患者與非過敏個案之 T-細胞抗原定位點與輔助性 T 細胞免疫分化偏向分析※

※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC 89-2314-B-002-279

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：胡忠怡

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：台大醫學院 醫事技術學系

中 華 民 國 90 年 12 月 31 日

行政院國家科學委員會專題研究計畫成果報告

家塵滿過敏原 Der p2 在塵滿過敏患者與非過敏個案之 T-細胞抗原定位點與輔助性 T 細胞免疫分化偏向分析

計畫編號：NSC 89-2314-B-002-279

執行期限：89 年 8 月 1 日至 90 年 7 月 31 日

主持人：胡忠怡　　執行機構及單位名稱: 台大醫學院醫事技術學系

E-mail address: cyhu@ha.mc.ntu.edu.tw

一、中文摘要

家塵滿是引起國人罹患過敏性疾病重要的致敏原，其中塵滿軀體蛋白 Der p2 是主要致敏原之一。雖然塵滿無所不在，但並非所有接觸過敏原的人皆會發生過敏反應，足見致敏原在過敏患者與非過敏個體引發不同形式的免疫反應。探討 Der p2 蛋白分子上的 T-細胞抗原定位點以及對塵滿發生反應的 T-細胞產生之細胞素 IFNr 及 IL4 是特別重要的研究課題。欲探究 Der p2 致敏原在有塵滿過敏性患者與無過敏疾患者主要的 T-細胞抗原定位點有否差異？而針對同一 T-細胞抗原定位點，在過敏患者與非過敏個案體內引發的主要免疫反應形式(Th1 或 Th2)是否有不同？以往利用定量受 Der p2 或其牲呔刺激產生的細細胞分裂代表細胞活化、或度量受活化細胞培養基中 T 細胞泌出的細胞素 IFNr 及 IL4 代表免疫反應進行 Th1 或 Th2 方向，卻無法精確解答上述疑問。本計畫利用合成的 Der p2 牲呔刺激受試者(11 例塵滿過敏患者與 12 例非過敏個案)之週邊血液單核細胞，並採用酵素吸附免疫色點分析法偵檢週邊血液 T 細胞對致敏原 Der p2 之精確 T-細胞抗原定位點。結果顯示 1.大部份個案於 Der p2 (p1-38)區域間至少有一個 T-細胞抗原定位點(Fig 2)，而位於此區間之 T-細胞抗原定位點因人而異。2. 多數狀況下,產生 IFNr 與產生 IL4 之 T-細胞抗原定位點一致，然而亦有少數例外情形。3. 比較過敏與非過敏個案組間對各別 Der p2 牲呔刺激後產生之 IFNr 級 IL4 色點數則發現在近 Der p2 (p1-38)片刻 C-端牲呔在兩受測組間有顯著的差異，其意義有待進一步探究。

關鍵詞：家塵滿、Der p2 過敏原、T 細胞抗原定位點、酵素免疫吸附色點分析

Abstract

Dermatophagoides pteronyssinus is the most important source of allergens that cause allergic disorders in northern Taiwan. Group II allergen of *Dp*., Der p2, was the major allergen of Dp. House dust mites are ubiquitous, however, not all subjects encounter HDM develop allergic disorders. It was of particular interest to look at the T-repertoire on major HDM allergens, investigating cytokine production profile of the HDM-sensitive individuals, and to tell their difference with that of the non-atopic subjects. Previous studies addressed the questions by measuring the proliferative responses as well as the cytokines IFNr/IL4 secreting into culture supernatant after stimulating human peripheral blood mononuclear cells or Der p2-specific T cell lines/T cell clones with Der p2 allergen or its peptides. Thus, Der p2-reactive T-repertoire and the immune deviation of these helper T cells could not be dissected in the single cell level. It is still unclear if the Der p2-reactive T-repertoire and the cytokine production profile of the Der p2-reactive T cells differ among non-atopic (NA) subjects and HDM-sensitive allergic (A) individuals. In this project, we tested 11 HDM-sensitive (A) and 12 non-atopic (NA) subjects for their T-epitopes on N-terminal of Der p2 allergen. Peripheral blood mononuclear cells were cultured in medium containing overlapping synthetic peptides of Der p2 for 20 hours and tested for IFNγ, IL4 producing cells (SFCs) by ELISPOT assay. Most (70-80%) of the subjects had at least one T-epitope in Der p2 (p1-38) region. The major T epitopes varied in different subjects. The IFNγ-producing epitope was comparable to the IL4–producing epitopes in most of the cases. Comparing the number of spot forming cells (SFCs) in response to different Der p2 peptides revealed significant differences, especially at the C-terminal of Der p2(p1-38), between A and NA subjects.

Keywords: House dust mite (HDM), Der p2 allergen, T-epitope, ELISPOT.

二、緣由與目的

In Taiwan, house dust mite (HDM) is the most important source of indoor allergens that cause atopic diseases such as allergic asthma, allergic rhinitis, and atopic dermatitis (1). The presence of allergen-specific IgE antibodies is implicated in sensitization of mast cells and the pathogenesis of unique inflammatory responses-associated allergic manifestations. A positive skin test to allergen correlates well with the presence of specific IgE, which was commonly used for clinical diagnosis in allergic diseases. Prevalence of allergic disorders, such as asthma (AS) and allergic rhinitis (AR) among school-age children in Taipei was reported to be 26.5% (2). Der p2 is the most important allergen that

cause allergic asthma in Northern Taiwan (3,4). Der p2-specific IgE could be detected in up to 80% or more of the sera from AS, AR patients. House dust mite antigens are ubiquitous, however, not all individuals exposed to HDM antigens developed allergy. It has been well known that the allergic reaction toward mite's proteins bias to Th2-type immune responses (5). The allergen-specific T cell lines derived from asthmatic children were of Th2-type, secreting IL4 but barely IFNr. Th2 lymphocytes enhance the B cells producing allergen-specific IgE antibodies. Activation of IgE-bearing mast cells or basophils through allergen binding triggers immediate hypersensitivity, results in releasing of mediators that attracting eosiniphils and polymorphonuclear cells, and cause inflammatory responses. For years, the allergens of HDM were identified consecutively. Those molecules were successfully cloned and recombinant allergen proteins Der p2, p5, and p8 can be produced for clinical and research use (6-9).

Previous studies of the T-epitopes on HDM allergens such as Der p2 were carried out by stimulating freshly isolated human peripheral blood mononuclear cells (PBMCs) or established Der p2-specific T cell lines (TCLs)/T cell clones (TCCs) with recombinant or synthetic Der p2 peptides, and detecting T cell activation by their proliferation (^{3}H-Thymidine incorporation) (10,11). It revealed that Der p2 allergen contained multiple T-epitopes and they differed from individual to individual (11, 12). There was no significant difference between the Der p2-sensitive allergic patients (A) and non-atopic control subjects (NA) (11-13). In studying the cytokine profile of the Der p2-reactive T cells, it showed that specific Der p2-specific TCLs or TCCs derived from Der p2-sensitive allergic patients secreted cytokines in a high IL4/IFNr ratio as compared to those derived from non-atopic control individuals (10, 14). However, proliferating response or level of secreted cytokine in the whole T cell culture could not indeed illustrate if the peripheral T-repertoire reactive to Der p2 differs between A and NA subjects. Based on the information obtained from bulk T cell culture, whether the T cells from A and NA subjects that bear same peptide specificity showed different cytokine production profile remains unclear. It is of disadvantage to use Der p2-specific TCLs or TCCs to assess the Der p2-reactive repertoire. Firstly, it was labor-intensive to maintain Der p2-specific TCLs or obtaining Der p2-specific TCCs. Secondly, we do not know for certain if all the T cells reactive to Der p2 were equally reactivated during the process of TCL re-stimulation. The actual T-repertoire of an individual might be skewed before we get started to assess it.

Heterogeneity of cytokine synthesis in polarized Th1 and Th2 population could be assessed at a single cell level by ELISPOT assay (15). This technique not only accesses the frequencies of PBMCs reactive to a given allergen or allergenic peptide, but can also assess the cytokine produced by each cell simultaneously. To access

1. Major peripheral T-repertoire on the major *Dp.* allergen Der p2, both on A and NA subjects.
2. The difference of cytokine production profile or immune deviation of the Der p2 peptide-reactive Th cells at the single cell level.

We stimulated PBMCs from 12 NA and 11 A (classified by presence of Der p2-specific IgE (ref.17) or not) subjects with overlapping synthetic peptides spanning Der p2(1-38) (Figure 1), which was reported to be a major T-epitope (Chua et. al., unpublished). These informations will be of value in mapping the potential allergenic T-epitopes on Der p2 molecule, which will be important in application designing peptide-based immunotherapy of HDM allergy. This system will be a powerful tool in studying mechanisms involved in a successful allergen-specific immuntherapy.

三、結果與討論

Most (70-80%)of the test subjects had at least one IFNr- or IL4-producing T epitope within the region Der p2 (p1-38)(Fig. 2). The major T-epitopes within this region varied between subjects. This might be attributed to different human leukocyte antigens that participated antigen-presentation steps in T cell activation. In most of the cases, the IFNr-SFCs-inducing peptides coincided with the peptides induce IL4-SFCs, which implied most T-epitopes elicited both Th1 and Th2 responses (Fig. 2A). However, we also found 3 atopic and 1 NA subjects who had diverse cytokine expression profile toward peptides analyzed (Fig. 2B). Therefore, it indeed happened the some T-epitopes could elicit Th1 or Th2 response exclusively. It was also interesting to find that IFN-SFCs and IL4-SFCs toward each Der p2 peptide differed significantly between the A and NA subjects, especially for peptides at at the C-terminal part of the Der p2 (p1-38).Both the IFNr-SFCs and IL4-SFCs among the atopic subjects were significantly higher than that of the atopic subjects. This phenomenon awaits further investigation.

四、計畫成果自評

We successfully utilized the ELISPOT assay system to investigate the fine map of T-epitopes on the N-terminal of the major HDM allergen, Der p2. It will be of use in studying and designing peptide-based immunotherapy of HDM allergy. This system will be a powerful tool in studying mechanisms involved in a successful allergen-specific immuno- therapy.

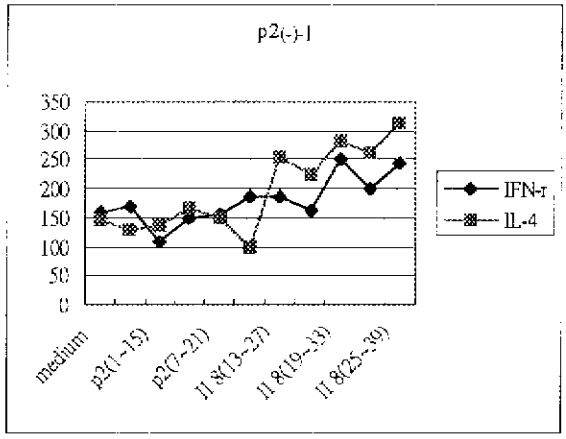
五、參考文獻

1. Hsieh KH A study of intracutaneous skin tests and radioallergosorbant tests on 1000 asthmatic children in Taiwan. Asian Pacific J Allergy Immunology 1984: 2: 56-60.
2. Hsieh KH, Tsai YT. Increasing prevalence of childhood allergic disease in Taipei, Taiwan, and the outcome. In: Progress in Allergy and Clinical Immunology. vol. Kyoto. ed. by Miyamoto T and Okuda M. Hogrefe & Huber Publisher. 1991: 223-5.
3. Tang RB, Tsai LC, Hung MW, Hwang B and Wu KG. Detection of house dust mite allergens and immunoblot analysis in asthmatic children. J Asthma 1988; 25: 83-8.
4. Lin KL, Hsieh KH, Thomas WR, Chiang BL and Chua KY. Characterization of Der p V antigen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. J Allergy Clin Immunol 1994: 94: 989-96.
5. Wierenga EA, Snoek M, DeGroot C, Christien I, Bos JD. Jansen HM, Kapsenberg ML (1990) Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. J Immunol 144: 4651-4656.
6. Lynch NR. Thomas WR, Chua KY, Garcia N, Di Prisco MC and Lopez R (1994) In vivo biological activity of recombinant Der P II allergen of house dust mite. Int Arch Allergy Immunol 105(1): 70-74.
7. Lin KL. Hsieh KH. Thomas WR, Chiang BL and Chua KY (1994) Characterization of Der P V antigen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. J allergy Clin Immunol 94: 989-996.
8. Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR (1990) Isolation of cDNA coding for the major mite allergen Der P II by IgE plaque immunoassay. Int Arch allergy appl Immunol 91: 118-123.
9. O'Neill GM, Donoran GR. Baldo BA (1994) Cloning and characterization of a major allergen of the house dust mite, Dermatophagoides pteronyssinus, homolougous with glutathione S tranferase. Biochemica et Biophysica Acta 1219:521-528.
10. Van Neerven RJJ, van t'Hof W, Ringrose JH, Jansen HM, Aalberse RC, Wierenga EA, Kapsenberg ML (1993) T cell epitopes of house dust mite major allergen Der p II. J. Immunol 151:2326-2335.
11. O'Hehir RE, Verhoef A, Panagiotopoulou E, Keswani S, Hayball J, Thomas WR, Lamb JR (1993) Analysis of human T cell responses to the group II allergen of Dermqtophagoides species: localization of major antigenic sites. J Allergy Clin Immunol 92: 105-113.
12. Wierenga EA, Snoek M, Jansen HM, Kapsenberg ML (1990) Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. Eur J Immunol 20:1519-1526.
13. Okano M, Nagano O, Kino K, Yasueda H, Baba Y, Saito C, Matsuda Y, Ohta N (1992) Population analysis of cellular responses to synthetic peptides of Der pII, a major allergen molecule of Dermatophagoides pteronyssinus, in allergic and non-allergic subjects. Allergy 49:436-441.
14. Hoyne GF, Bourne T, Kristensen N, Hetzel C, Lamb JR (1996) From epitope to peptide to immunotherapy. Clin Immunol and Immunopathol 80:S23-30.
15. Openshaw P, Murphy EE, Hosken NA, Maino V, Davis K and Murphy K (1995) Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 population. J Exp Med 182:1357-1367
16. Czerkinsky CC, Nilsson LA, Nygren H, Ouchterlony O, Tarkowski A (1983) A solid-phase enzyme-linked immunospot (ELISPOT) assay for eneumeration of specific antibody-secreting cells. J Immunol Methos 65:109-121.
17. Hu CY, Hsu PN, Lin RH, Hsieh KH, Chua KY (2000) HLA DPB1*0201 allele is negatively associated with IgE responsiveness specific for house dust mite allergens in Taiwan. Clin Exp Allergy 30: 538-545.

Figures and tables:

Figure2 ELISPOT/ 10^5 PBMCs responsive to Der p2 peptides.

A. a non-atopic subject (Derp2-specific IgE negative)



B. an atopic subject (Der p2-specific IgE positive)

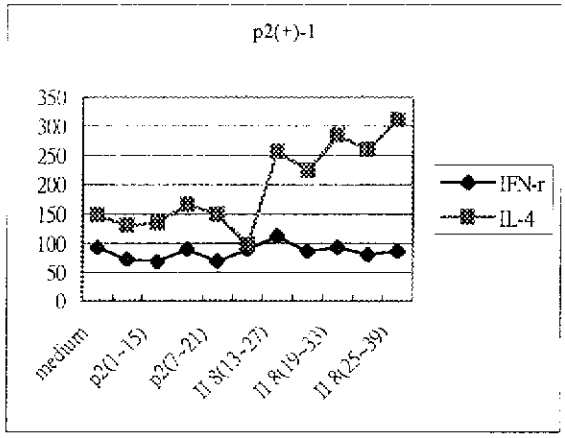


Figure 1 Der p2 amono acid sequence of 2 isoforms and the peptide used in this study:

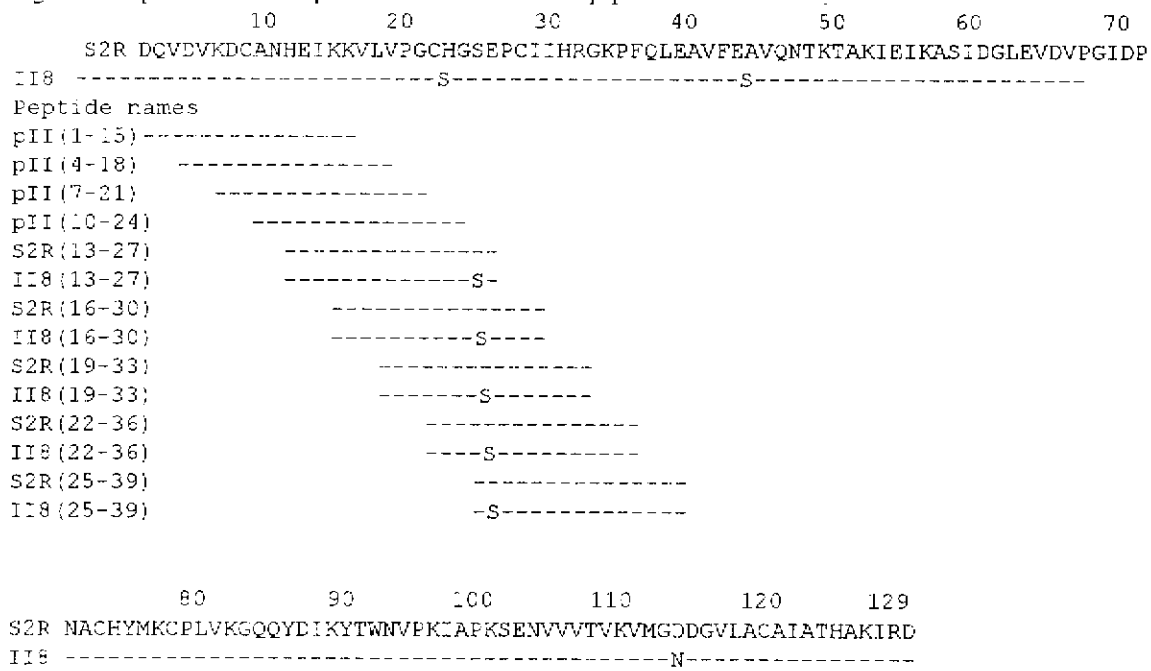


Table1 Spot forming cells (SFCs) per 10^5 PBMCs in response to Der p2 peptide stimulation

each 10^5 MNCs	Der p2-specific IgE (-) n=12	Der p2-specific IgE (+) n=11	Mann-Whiteney U test p value
IFNg SFCs			
medium	152(79~218	84(7~319)	
Der-p2(II 8)	13698~176)	72(11~277)	
Der-p2(S2R)	130(45~194)	66(13~284)	
p2(1~15)	124(51~216)	52(13~334)	
p2(4~18)	144(64~263)	71(6~327)	
p2(7~21)	138(70~186)	48(3~306)	
p2(10~24)	122(53~222)	56(10~295)	
S2R(13~37)	128(65~190)	56(7~287)	
II 8(13~27)	129(37~218)	56(5~242)	
II 8(16~30)	138(74~191)	60(4~196)	
S2R(19~33)	132(58~211)	71(2~254)	
II 8(19~33)	130(58~250)	52(2~224)	0.032
S2R(22~36)	145(68~232)	73(4~221)	0.033
II 8(22~36)	154(71~235)	76(4~278)	
S2R(25~39)	152(78~264)	60(5~203)	0.007
II 8(25~39)	158(82~259)	72(4~197)	0.012
IL4 SFCs			
medium	137.5(42~207.5)	61(5~296)	
Der-p2(II 8)	132.25(92~200)	65(6.67~302)	
Der-p2(S2R)	166(119~213)	56.5(6~268)	0.01
p2(1~15)	141(107~256.5)	43(2.67~350)	
p2(4~18)	142(106~221.5)	77.5(1~309.5)	
p2(7~21)	133.75(92~243)	44(1~304.5)	
p2(10~24)	151.5(82~267)	51(4.33~304.5)	
S2R(13~37)	152.25(70~210.5)	69.5(3.67~263)	
II 8(13~27)	158.5(77~274)	46.25(3.33~174.5)	0.001
II 8(16~30)	161.75(94~284)	33.5(5.33~200)	0.0004
S2R(19~33)	177(86~301)	64.75(2.67~152)	0.0000092
II 8(19~33)	166(58~289)	60.25(4~194)	0.0024
S2R(22~36)	151.5(103~266)	64.5(3~182.5)	0.001
II 8(22~36)	178.75(95~260)	60.75(2.33~160)	0.0003
S2R(25~39)	164(45~274.5)	52.25(2.67~170)	0.0005
II 8(25~39)	177.5(111.5~311)	65(4.33~177.5)	0.0005