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Comparative analysis of the Group V allergens from house dust mite-*Dermatophagoides pteronyssinus*

家塵瑞第五組過敏原之比較分析

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中文摘要

家塵瑞第五組過敏原是氣喘病重要的因子之一，進一步了解其過敏性決定點將有助於新策略的研發及更有效治療方法的提出，本研究繼選殖 Der p 5 基因之後，另找到五個相關全長的基因，並進行 DNA 排列順序分析，得其有多個位置變異，並在第六十一個氨基酸的位置，由苯丙氨酸(Analine)換成麩氨酸(Glutamine)，後續將更研究台灣環境中家塵瑞之第五組過敏原基因的變異性，以提供過敏免疫決定點的分析。

關鍵詞：家塵瑞，第五組過敏原，多樣性，抗原性的分析

Abstract

The house dust mite allergens, Der p 5, are the important allergic factor in asthmatic diseases. The understanding of allergenic determinant will provide a new strategy for exploring the potential efficacy of various therapeutic interventions. Five clones of Der p 5 were obtained from cDNA library of *Dermatophogoides pteronyssinus*. Sequence data obtained from cDNA clones revealed a significant variation of GCC(Ala) to GAA(Glu) at residue 61. The results of this study were to analyze sequencing data on recombinant Der p 5 and to extend the recombinant work by determining the sequence polymorphisms between the different recombinant clones from enviremetal mite in Taiwan. It will be useful to analyze the T and B cell determinants of allergens.

Keywords: House dust mite, Der p 5 Allergens, Polymorphism, Antigenicity

Introduction

House dust mite is well recognized as a major factor in the development of allergic

diseases, such as asthma, allergic rhinitis and atopic dermatitis (1). Mites of the *pyroglyphidae* family including *Dermatophogoides pteronyssinus* (Dp), *Dermatophogoides farinae* (Df)and *Euroglyphus maynei* are the main sources of mite allergens in house dust. The predominant mite species found in Northern area of Taiwan(2), Australia, New Zealand, the United Kingdom and Western Europe is (Dp)(3), while *Dermatophagoides farinae* (Df)is a contribution in many areas of United States, Japan,and Continental Europe. Both these species of mite sensitize between 10 and 20% of the general population (4,5), eliciting both humoral and cellular responses. Another glyciphagid mites, which is widespread in house dust in the tropics is *Blomia tropicalis*(6). In Singapore, B. tropicalis allergen could ber detecdted in 87% of homes and 70% of schools (7) whilst in Cartagena, Columbia, *B. tropicalis* was seen in 96% of floor and mattress dust samples and was even more prevalent than *D. pteronyssinus* (6). In this regard, a many studies have been performed to identify the allergens of predominant mites produce.

The house dust mite allergens, Der p 5, in *Dermatophagoides pteronyssinus* are the important determinants in allergeic diseases. Sequence data obtained from cDNA clones revealed a significant degree of sequence variation. The aims of this study at firstly to provide immunochemical and sequencing data on both the native and recombinant Der p 5 proteins and; secondly developing polymerase chain reaction (PCR) techniques to obtain Group 5 sequences from cultured and environment mites at the genomic level.

Material and Methods

Collection of mite body

Cultured mite were purchased from Commonwealth serum Laboratory Ltd. (CSL), Melbourne, Australia. Environmental mite isolates were collected from the dust sample obtined from various parts of Australia and Taiwan,mite were separated by Flotation on saturated NaCl and 30% sucrose solution. They were then cultured in dessicated liver powder(oxoid) at 25 °C and 75% humidity. Dried mite were also been used in the study.

Purification and N-terminal sequence of native Der p 5 allergen

In order to purify native Der p 5, the crude mite extract was subjected to immunoaffinity chromatography employing the anti-Der p 5 antibody (5 mg/ml CNBR-sepharose). After two extensive washing steps with 0.01 M benzamidine, 5%(v/v) glycerol, 0.05 M HEPES (pH 7.4) supplemented with 0.1 M NaCl and I M NaCl, respectively. Der p 5 was eluted with 3 M KSCN in the same buffer, Der p 5 containing fractions was polled and stored at –70°C in 5 %(v/v) glycerol, 0.1 M NaCl, 50 MM HEPES (pH 7.4).

Sequencing of the cDNA of Der p 5

Sequence polymorphism studied of Der p 5

was carried out at both cDNA and genomic DNA level. cDNA clones coding for different isoforms were first obatained by scteening cDNA library by IgE plaque radioimmunoassy and DNA hybrifization,and then subjected to DNA sequencing by dideoxynucleotide chain termination method using sequenase Kit. The sequences of the 5* and 3* primers for DNA amplification are 5*AAAAAGATCTATCATGAAATTCATC-3 (Bgl II site underlined) and 5*-ATTAAGCTTAACTTCAATCTTTTT-3* (Hind III site underlined), respectively, encompassing the entire Der p 5 sequence. Genomic DNA coding for Der p 5 obtained by PCR amplification from either purified mite total genomic DNA or directly from crude DNA prepared from dried mites.

Results

Dep 5 related clones

The first report on Der p 5 described a λgt 11 cDNA clone for Der p 5 which produced a fusion protein which reacted with IgE. The additional clones WL and WM coding for Der p 5 were obtained in this study. The sequence for the Der p 5 allergen is now defined except for the exact N-terminal which will need to be determined from the natural protein. Using immunoblot analysis, the native antigen of Der p 5 (nDer p 5) could be detected in house dust with sandwich immunoassay.

Analysis for cDNA and genomic DNA polymorphism of Der p 5

Five cDNA clones were obtained by IgE plaque immunoassay and DNA hybridization. Table 1 demonstrated the nucleotide variation of Der p 5 clones. We have now refined protocol to amplify Der p 5 sequences from a single live mite. In short, a PCR-based technique has now been established to facilitate the study of variants of major mite allergens from different geographical regions. The sequence polymorphism of PCR products were then analysed by DNA sequencing.

Table 1: Sequence variation of Der p 5 clones.

Clone	residues with polymorphic codon			
	11	21	61*	82
WL	GCC	GAT	GCC	ATT
WM	GCA	GAT	GAA	ATC
L25	GCC	GAA	GCA	ATT
C2A	GCC	GAT	GCC	ATT
C2B	GCA	GAT	GAA	ATC

*mutation of GCC(Ala) to GAA(Glu)

Discussion

It has been demonstrated by Dr. Thomas et al. That different Der p 1, Der 2, and Der p3 cDNA clones contain significant differences at their nucleotide level in the epitope regions(8,9,10). According to literature review, it is clear that isoallergenic variation is not limited to mite allergens, but can also be found in the major birch pollenallergens(11),in the ragweed allergen Amb a 1 (12), and in group 5 allergen from temperature grasses(13). These findings showed that

serum IgE from different birch allergic patients exhibits distenct isoform reactivity patterns in 2D gel electrophoretic systems, suggesting that amino acid substitutions in these isoallergens alter IgE epitopes(14) and T cell responsiveness (11). Previous experimental observations suggest that multiple isoallergens play an important role in the sensitization to environmental allergens(15,16,17,18). Therefore, this investigation will be useful to modulate the T and B cell determinants of allergens and that may provide a new strategy for exploring the potential efficacy of various therapeutic interventions.

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Evaluation

While some polyphysium of Der p 5 has been obtained in this study, there are a number of other clones and PCR product which require to be more studied at gene sequence. Further work, we holp to provide more information for understanding of the primary structure of isoforms of mite allergen-Der p 5.