ABSTRACT

The prevalence of house dust mite (HDM) allergy was determined among the Malaysian population with emphasis to three main species, *Dermatophagoides pteronyssinus*, *D. farinae* and *Blomia tropicalis*. Adult patients with allergy-related diseases, (namely allergic rhinitis and asthma), normal healthy subjects, and asthmatic children were studied for HDM sensitization. In this study, the *in vivo* skin prick test (SPT) was tested on 291 allergic rhinitis and 298 asthmatic patients from the University Malaya Medical Centre, Kuala Lumpur and 30 normal healthy subjects. Of the allergic rhinitis patients, 76% reacted to *D. pteronyssinus* while 72% reacted to *D. farinae*. In the asthmatic group, 80% reacted to *D. pteronyssinus* and 74% reacted to *D. farinae*. In addition, the storage mite, *B. tropicalis* also produced positive SPT results in 66% of the allergic rhinitis patients and 73% in the asthmatics. In allergic rhinitis patients, SPT also revealed sensitivity to other aeroallergens: house dust (40%), cockroach (52%), pollen (10%) and fungal spores (11%). Adult asthmatics also showed higher percentage of reaction to almost all the aeroallergens in SPT, predominantly to HDM species. In addition, seafood also produced quite high percentage of positivity (~30%) in SPT. In 150 asthmatic children, 90% had sensitisation to at least one of the three HDMs and 65% had specific-IgE to all three HDMs. Interestingly, D. *farinae* affected the children more than *D. pteronyssinus;* affecting 85%, followed by *D. pteronyssinus*-specific IgE in 75%, while 74% had IgE against *B. tropicalis*. Monosensitization was detected in 18 (12%) of the children mostly to *D. farinae*. Dust samples from mattresses were found to harbour more mite allergens than the floor and 56% of the number of mattresses studied had Der p 1 compared to 33%, which had Der f 1.

Mite tropomyosin (Der p 10) was selected for its recombinant production and its epitope mapping. The recombinant Der p 10 was obtained by molecular cloning technique through the *E. coli* and the yeast *Pichia pastoris* expression systems. Through sequencing, the deduced amino acid sequence was found to have 98% homology with that of the native Der p 10. *E. coli* expression system produced 1.0 mg/ml purified recombinant GST-Der p 10, whereas the *Pichia* expression system yielded 0.54 mg/ml of protein. *Pichia*-produced Der p 10 showed significant immunogenicity in mice, producing antiserum that could recognize the Der p 10 component in the crude extract. Specific IgG-binding reactivity of the mouse antiserum to these recombinants of Der p 10 could be traced significantly in crude mite extracts and various commercial tropomyosin-containing allergen extracts suggesting that these recombinants of Der p 10 had similar IgG recognition sites as those

found in the native Der p 10 in mite total extracts and other tropomyosin-containing allergens. These Der p 10 recombinants also proved their antigenicity as they were recognizable by shrimp tropomyosin-specific IgG antibody, suggesting the similarity of epitopes on the recombinants to those found on a native tropomyosin protein. *In vivo*, both recombinants also proved functional, eliciting positive reactions comparable with that of crude extracts from tropomyosin-containing allergenic sources such as shrimp and lobster. Subsequently, when tested with ELISA by using sera of Malaysian allergic patients, IgE-binding reactivity with a frequency of about 20-30% was obtained; comparable in frequency and binding strength as observed in 60 Taiwanese allergic patients. This however reflected the failure of these recombinants to emulate the high binding reactivities shown by native tropomyosin (80%) as previously reported.

An investigation of IgE-binding reactivities involving the cloned GST-Der p 10 with another mite tropomyosin, GST-Blo t 10, revealed a high correlation of allergenicity in the allergic subjects. However, low response to Group 10 mite allergens shows that it is not a major allergen in Malaysia. The subsequent evaluation of IgE-binding reactivities involving various recombinant allergenic components in the HDMs, was able to reveal the component-resolved allergenicity, albeit incomplete, in the allergic subjects. Group I and 2 mite allergens, (Der p 1/ Blo t 1 and Der p 2/Blo t 2), identified majority of the sensitized subjects and comparable importance of Group 5 allergens was also discovered.

Both linear and conformational peptides of Der f 10 were synthesized as non-cleavable peptides on pins using the Multipin Peptide Synthesis technique. Subsequently, they proved to be reactive when tested in a modified pinELISA. Pepsacn showed IgE binding at four prominent regions representing the location of B-cell epitopes on Der f 10. Finer mapping using smaller 5-mer peptides confirmed the previously predicted location of epitopes whereby 4 immunodominant epitopes were identified on this allergen; at EVRAL, LQKEV, VDRLE and EDELV showing over 75% IgE-binding reactivity while chimeric peptides identified immunodominant Der f 10 epitopes at KEARMMAEDADRKYDE, ITDEERMDGLENQLKE, EDADRKYDEVARKLAM, DEVARKLAMVEADLER, ERAEERAETGESKIVE and ETGESKIVELEEELRV. Similarly, another allergen, Blo t 5, also constructed as linear synthetic 12-mer peptides overlapping by 5 amino acids, showed distinctive IgE binding reactivities at 4 common regions; at residues 1-12, 56-72, 86-97 to 126-134 of the Blo t 5 protein.

ABSTRAK

Prevalens alergi hama rumah dikenalpasti di kalangan populasi Malaysia dengan memberi penekanan kepada 3 spesis utama, *Dermatophagoides pteronyssinus*, *D. farinae* dan *Blomia tropicalis*. Penghidap-penghidap rhinitis alergi and asma, subjek-subjek normal, dan kanak-kanak (2-15 tahun) telah dipilih untuk kajian ***sensitization*** terhadap hama rumah. Ujian tusukan kulit (SPT) yang dijalankan melibatkan 291 penghidap rhinitis dan 298 penghidap asthma dari Pusat Perubatan Universiti Malaya, Kuala Lumpur. Hanya 30 subjek normal telah menjalani ujian SPT secara sukarela. Di kalangan penghidap rhinitis alergi, 76% menunjukkan reaksi terhadap *D. pteronyssinus* manakala 72% daripada mereka menunjukkan reaksi terhadap *D farinae*. Dalam kumpulan penghidap asma pula, 80% bereaksi terhadap *D. pteronyssinus* dan 74% terhadap *D farinae*. Selain daripada itu, hama *B. tropicalis* juga menghasilkan keputusan yang positif pada 66% penghidap rhinitis alergi dan 73% di kalangan penghidap asma. Melalui SPT, penghidap rhinitis alergi turut menampilkan kesensitifan terhadap pelbagai alergen lain seperti habuk rumah (40%), lipas (52%), debunga (10%) dan spora kulat (11%). Penghidap asma dewasa turut juga menunjukkan reaksi yang hampir sama. Selain daripada itu, alergen daripada makanan laut seperti ketam, tiram dan udang turut menghasilkan peratus respons SPT-positif yang agak tinggi (~ 30%). Dalam kajian yang melibatkan 150 orang kanak-kanak penghidap asma, 90% daripada mereka adalah sensitif terhadap salah satu daripada hama dalam kajian ini. Daripada bilangan ini, 65% didapati mempunyai IgE-spesifik terhadap ketiga-tiga spesis hama ini. Yang menariknya, *D. farinae* lebih banyak didapati memberi kesan terhadap kanak-kanak (85%) diikuti dengan *D. pteronyssinus* (75%) dan *B. tropicalis* (74%). Kesensitifan tunggal turut dikesan dalam 18 (12%) daripada kanak-kanak ini; kebanyakannya terhadap *D. farinae*. Lebih banyak alergen hama didapati dalam sampel habuk tilam berbanding dengan habuk lantai. Alergen Der p 1 didapati daripada 56% daripada tilam-tilam yang dikaji dan Der f 1 pula dikesan pada 33% tilam-tilam itu.

Di kalangan komponen-komponen protein penyebab alergi pada hama-hama ini, tropomiosin telah dipilih untuk penghasilan rekombinannya dan untuk pemetaan epitopnya. Tropomiosin hama rumah rekombinan (Der p 10) diperolehi melalui teknik pengklonan molekular diikuti dengan sistem pengekspresian bakteria *Escherichia coli* dan yis *Pichia pastoris*. Melalui teknik penjujukan DNA pula, Der p 10 yang dihasilkan oleh *E. coli* didapati mempunyai 98% tahap homologi jika dibandingkan dengan Der p 10 asli. Sistem pengekspresian *E. coli* menghasilkan 1.0 mg/ml rekombinan GST-Der p 10 manakala sistem pengekspresian *Pichia* pula menghasilkan 0.54 mg/ml protein. Der p 10 yang dihasilkan oleh *Pichia* ini menunjukkan ciri keimunogenan yang signifikan pada mencit, dan turut menghasilkan antiserum yang dapat mengecam komponen Der p 10 dalam ekstrak hama yang

asli. Der p 10 rekombinan ini juga dapat dicam oleh antiserum yang dihasilkan terhadap tropomiosin udang. Ini secara tidak langsung menunjukkan bahawa protein rekombinan yang terhasil mempunyai ciri-ciri yang menyerupai tropomiosin asli. Kedua-dua rekombinan Der p 10 ini mampu mengesan 20-30% sampel serum Malaysia yang positif dan keputusan hampir serupa dapat diperhatikan di kalangan 60 penghidap alergi Taiwan. Akan tetapi, walaupun protein-protein rekombinan ini didapati bersifat antigenik, ciri kealergenan tinggi yang dipertunjukkan oleh Der p 10 asli gagal dipamirkan jika dibandingkan dengan kemampuan Der p 10 asli yang dilaporkan dapat mengesan 80% tahap alergi dalam individu-individu beralergi.

Penyiasatan ke atas kereaktifan pengikatan IgE yang melibatkan GST-Der p 10 dan GST-Blo t 10, telah berjaya memperlihatkan korelasi ciri allergenik kedua-dua tropomiosin ini di kalangan subjek-subjek beralergi. Akan tetapi, respons yang rendah terhadapnya menunjukkan bahawa kumpulan alergen ini bukanlah suatu alergen yang bersifat major di kalangan penghidap alergi tempatan. Penilaian seterusnya berdasarkan kereaktifan ikatan IgE yang melibatkan pelbagai komponen alergenik dalam hama yang dihasilkan secara teknik rekombinan, telah berjaya mendedahkan kealergenan hama secara terperinci, walaupun tidak lengkap. Alergen hama Kumpulan 1 (Der p 1/Blo t 1) dan 2 (Der p 2/Blo t 2) telah berjaya mengenalpasti kebanyakan subjek-subjek yang menerima kesan alergen-alergen ini. Alergen hama Kumpulan ke-5 turut didapati memberi kesan yang penting dalam alergi.

Kedua-dua peptida linear dan berkonformasi bagi Der f 10, telah disintesis pada pin-pin melalui teknik 'Multipin Peptide Synthesis' dan diuji dengan teknik ELISA yang diubahsuai untuk mengesan antibodi IgE spesifik pada sampel serum sekumpulan penghidap-penghidap alergi. Kereaktifan pengikatan IgE berfrekuensi tinggi berupaya mendedahkan lokasi-lokasi yang berkemungkinan tinggi menempatkan epitop-epitop sel-B. Pemetaan rapi ke atas epitop-epitop yang disyaki tadi telah mengenalpasti 4 epitop yang dominan secara immunogenik iaitu EVRAL, LQKEV, VDRLE dan EDELV, yang menunjukkan lebih daripada 75% kereaktifan pengikatan IgE. Peptida-peptida kimerik yang didapati reaktif pula ialah KEARMMAEDADRKYDE, ITDEERMDGLENQLKE, EDADRKYDEVARKLAM, DEVARKLAMVEADLER, ERAEERAETGESKIVE dan ETGESKIVELEEELRV. Allergen Blo t 5 dalam bentuk peptida sintetik linear 12-mer dengan pertindihan 5 amino asid telah dapat mengesan 4 jujukan yang berkemungkinan menempatkan epitop-epitop iaitu di amino asid pertama hingga ke-12, ke-56 hingga ke-72, ke-86 hingga ke-97 dan ke-126 hingga ke-134 pada jujukan protein Blo t.