



UNIVERSITI PUTRA MALAYSIA

**IDENTIFYING ROLE OF IMMUNE DYSREGULATION IN
MITE-INDUCED ALLERGIC RHINITIS AMONG MALAYS**

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FPSK(p) 2012 11

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By

PEYMAN AMINI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

February 2012

DEDICATION

**Dedicated especially to my mother and my brothers Masoud and Keyvan for their
unconditional love and support which allowed me to complete my studies**

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Maha Abdullah, PhD

Faculty: Medicine & Health Sciences

Allergic Rhinitis, the chronic allergic inflammation of nasal mucosa is associated with decreased learning, performance and productivity at work and school, as well as a reduced quality of life. Allergic Rhinitis is a major problem in Malaysia with up to 15% of the adult population affected by this disease. The most common causes for allergic rhinitis in Malaysia are house dust mites, cat fur, dog hair, moulds and cockroach. Established treatment protocols are available for allergic rhinitis. These protocols are mostly based on severity of the disease. However, selection of a proper therapeutic protocol for the patients is not straightforward and as widely acknowledged, the personal burden of illness as experienced by the allergic rhinitis patients cannot be fully assessed by standard clinical symptoms and signs which only

moderately correlate with patients' perceptions. Allergy and asthma are inflammatory diseases, caused by dysregulated immune responses in the respiratory mucosa. Although it is clear that Th2-driven immune responses critically effect the development of allergies, the role of upstream immunological regulatory mechanisms (e.g regulatory T cell activity) that develop *in vivo* to prevent Th2-driven inflammation and prevent allergic symptoms from developing in non-allergic individuals are not clear. This study aims to elucidate the involvement of two main mechanisms in immunology that controls allergic rhinitis: i) diversity in HLA class II genes and the expression of the corresponding T cell receptor V beta gene in regulatory T and effector T-cell populations and ii) the phenotype of the regulating T cell populations important in preventing the symptoms, by comparing with normal individuals. These parameters were also tested as potential markers to predict disease severity. One hundred and sixty four allergic rhinitis patients confirmed by using relevant clinical criteria and skin prick test were recruited into the study. A semi-assisted questionnaire was used to quantitatively evaluate the severity of the symptoms in patients. From the above, 57 adult Malays with mite-induced AR were further tested using laboratory experiments. Sixty healthy non-allergic individuals from the same ethnic background were also entered into the study as control group. Serum from blood samples was also collected to determine serum total and allergen (mite) specific IgE concentration. Flow cytometric immunophenotyping of PBMCs was performed to determine the expression of cell markers and cytokines associated with regulatory T cells (before and after culture and after specific stimulation with purified mite allergen). Specific T-effector

(the main contributing T cell type in pathology of allergic diseases) and the total regulatory T cell population (which prevent symptoms and are higher in healthy individuals) were isolated from the cultured cells using a FACS sorter. Sorted samples were semi-quantified for the expression of TCR-V β gene segments using RT-PCR method. MHC class II polymorphism was determined using polymerase chain reaction-sequence specific primer (PCR-SSP) method. All results were analyzed using statistical analysis software. Demographic and severity-related data derived from questionnaire showed that approximately 60% of allergic rhinitis patients can be classified as “moderate” sufferers. The results from *in vivo* (prick test) and *in vitro* (serum IgE) diagnostic methods did not show a significant correlation with symptom severity. Serum levels of specific IgE for *Dermatophagoides farina* and to a lesser extent, *Blomia tropicalis* showed positive associations with size of wheal in skin prick test. Polysensitive state of the disease to the mentioned 3 mite species was not a contributing parameter for having a more severe clinical profile. Other disease-specific data which were collected by using the questionnaire (e.g. the concurrent presence of other types of allergy or previous history of allergy) were also not correlated to the disease severity. HLA typing showed that DR7 may have a protective role against mite-induced allergic rhinitis in Malays; while 2.6% of allergic rhinitis patients expressed this allele, its frequency in control group was 14.2% (p -value=0.003). Also from 24 TCR-V β alleles studied, the expression of V β 18 on unstimulated effector T cells was significantly higher in allergic rhinitis (p -value=0.015) patients. Allergen-specific stimulation caused this prominent allele to shift to V β 7 (p -value=0.006).

However, the expression levels of the alleles did not show a significant correlation with severity-related parameters, suggesting that quantitating expression levels of the alleles may not be useful as a method for evaluation of severity in mite-induced AR. In regulatory T cells, no difference in expression of the alleles was observed between allergic rhinitis and control groups. Immunophenotyping results showed that the percentages of IL-10 and CD152 expressing subclasses were significantly higher in the control group compared to allergic rhinitis patients (p -values=0.016 and 0.013 respectively). The frequency of other regulatory T cell subsets was similar when compared between allergic and normal individuals. However, cell culture and especially mite allergen-specific stimulation elicited a higher percentage in most of the regulatory T cell subsets in the non-allergic group (p -values<0.05). Similarly, the correlation between the mentioned frequencies and severity parameters was significantly enhanced after *in vitro* stimulation of cells. In conclusion, the results of this study identified a protective effect from allergic rhinitis in individuals carrying the HLA-DR7 gene in Malay ethnicity. The dominant presence of T-effector cells expressing TCR-V β 7 & 18 alleles in allergic rhinitis patients is suggestive of an important role for these gene segments in regulating damage and highlights the significance in the interaction of HLA-TCR complexes in allergic rhinitis. The results of the immunophenotyping experiments emphasize the role of immune-dysregulation in the mechanism of allergic rhinitis. Comparative and correlative analysis of clinical and laboratory data indicates that, while most of the mentioned laboratory methods may be suitable for diagnosis of allergic rhinitis, *in vitro* stimulation and

immunophenotypic evaluation of regulatory T cells (especially IL-10 and CD152 expressing subclasses) may be considered as the more reliable approach for evaluation of the severity in this group of disease. The results of this study however, did not support the value of conventional diagnostic methods for evaluation of severity in allergic rhinitis in Malay ethnic group.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGENALPASTIAN PERANAN DISREGULASI IMUN DALAM ELERGI
RHINITIS DIKALANGAN BANGSA MELAYU**

Oleh

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Februari 2012

Pengerusi: Prof. Madya. Maha Abdullah PhD

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Alergi radang hidung (ARH), sejenis radang kronik pada mukosa hidung dikait dengan kerosotan dalam kecerdasan pembelajaran, pencapaian and produktiviti di sekolah dan kerja serta pengurangan kualiti hidup. ARH merupai salah satu masalah utama di Malaysia di mana 15% daripada populasi dewasa menghidap penyakit ini. ARH yang paling biasa di Malaysia ialah jenis perenial (sepanjang tahun), di mana penyebab-penyebab utama adalah hama habuk rumah, bulu kucing, bulu anjing, kulat dan lipas. Alergi dan asma adalah penyakit radang yang disebabkan oleh disregulasi sistem imun pada mukosa sistem pernafasan. Walaupun telah jelas tindakbalas imun jenis Th2 merupakan langkah kritikal dalam menentukan perkembangan alergi, mekansima awalan (seperti aktiviti sel T kawalan) yang menahan gejala (symptom) alergi dari

berkembang di individu tidak beralergi masih tidak jelas. Juga diakui, beban penyakit pada pesakit masih belum dapat dinilai secara penuhnya melalui tanda dan gejala klinikal yang hanya dapat dikait secara sederhana dengan persepsi keterukan penyakit ini pada pesakit. Parameter imun berpotensi digunakan untuk menentukan keterukan gejala penyakit alergi. Kajian ini bertujuan menentukan peranan dua mekanisme utama dalam sistem imun yang mengawal pembentukan ARH iaitu i) kepelbagaian molekul dalam HLA kelas II dan tahap ekspresi segmen gen Vbeta pada reseptor sel T, ii) pemilihan populasi sel T kawalan dan sel T efektor, melalui perbandingan dengan individu sihat. Parameter-parameter ini juga telah dikaji untuk kesesuaiannya sebagai penanda menentukan kadar keterukan gejala penyakit. Seramai seratus enam puluh empat pesakit ARH yang dikenalpasti dengan menggunakan kriteria klinikal dan ujian 'Skin Prick' telah mengambil bahagian. Satu soal selidik dibantu telah diisi oleh peserta untuk menentukan tahap keterukan gejala alergi pada pesakit. Dari jumlah tersebut, 57 sampel darah telah dikutip dari kalangan peserta Melayu untuk kajian makmal seterusnya. Seramai 60 sampel dari individu sihat bangsa Melayu juga disertakan selepas pengecualian individu yang menghadapi alergi yang lain disamping kriteria pengecualian yang lain. Sampel darah dari peserta-peserta ini diproses untuk pengasingan sel mononuclear darah periferi. Serum darah juga diperolehi untuk penentuan IgE 'total' dan paras IgE 'specific' kepada hama habuk. Semua prosedur sel kultur telah dioptimakan untuk perangsangan dengan protin tulin daripada hama. Fenotip T sel kawalan dikenalpasti dengan penanda sel dan jenis sitokin yang dihasilkan melalui penggunaan flow sitometri. Populasi T sel efektor (yang

menyebabkan gejala alergi pada pesakit) dan T sel kawalan (yang menahan gejala dan lebih tinggi antara individu pesakit) kemudian diasingkan dari kultur dengan alatan flow 'sorter'. Sel yang telah diasingkan kemudiannya dikaji untuk penentuan ekspresi gen segmenTCR Vbeta melalui cara RT-PCR. Kepelbagaian molekul MHC kelas II telah dikenalpasti melalui cara PCR-SSP. Data yang diperolehi telah dianalisa secara statistic untuk menentukan kaitan antara hasil ujian diagnostik dan kajian makmal (secara *in vivo* dan *in vitro*) dengan gejala keterukan alergi. Demografi dan data berkenaan keterukan gejala alergi menunjukkan 60% pesakit ARH boleh dikelaskan sebagai penghidap "sederhana". Hasil ujian diagnostik *in vivo* ('Skin Prick') dan *in vitro* (serum IgE) menunjukkan tiada korelasi antara kedua-dua ujian ini dengan gejala keterukan. Paras serum IgE spesifik kepada *Dermatophagoides farina* dan kurang ketara dengan *Blomia tropicalis*, didapati berkorelasi secara positif dengan saiz "wheal" yang diukur melalui ujian "skin prick". Ciri polisensitif pesakit terhadap ketiga-tiga spesis hama yang tersebut tidak merupakan parameter untuk mengukur profil klinikal yang lebih teruk. Ciri-ciri data klinikal yang lain yang diperolehi melalui soal selidik (iaitu menghidap penyakit alergi yang lain secara serentak atau sejarah alergi yang lalu) juga tidak berkorelasi dengan keterukan penyakit. Pentipan HLA telah mengenalpasti DR7 sebagai molekul yang dapat memberi perlindungan dari kejadian alergi terhadap hama habuk dikalangan masyarakat Melayu. Sementara 2.6% pesakit AH mengekspresi alel ini, sebanyak 14.2% (nilai- $p=0.003$) telah diperhatikan dikalangan individu yang sihat. Juga dari 24 TCR-Vbeta alel yang dikaji, ekspresi Vbeta18 pada sel T efektor, yang tidak dirangsang dalam culture, lebih tinggi diantara

pesakit ARH (nilai- $p=0.015$). Rangsangan khusus dengan *Der.p1* (hama habuk) telah memindahkan penonjolan alel ini kepada yang lain iaitu Vbeta7. Walaubagaimanapun, kedua-dua alel ini tidak menunjukkan kaitan signifikan dengan sebarang gejala keterukan alergi. Juga, dalam populasi sel T kawalan, tiada perbezaan dalam ekspresi alel-alel TCR-Vbeta diperhati antara pesakit ARH dan individu sihat. Imunofenotip sel T kawalan yang belum dikultur, menunjukkan peratusan yang lebih tinggi untuk subkelas IL-10+ dan CD152+ antara individu sihat berbanding pesakit ARH (nilai- $p=0.016$ dan 0.013 , masing-masing). Tiada perbezaan diperhatikan dalam kekerapan subkelas sel T kawalan antara dua kumpulan kajian ini. Walaubagaimanapun, setelah sel dikultur dan terutamanya selepas rangsangan khusus dengan hama habuk, didapati peningkatan sel T kawalan untuk kebanyakan subkelas jauh lebih tinggi dikalangan individu sihat (nilai- $p < 0.05$). Juga didapati korelasi antara kekerapan tersebut dengan gejala keterukan alergi, menambah setelah rangsangan sel secara *in vitro*. Sebagai kesimpulan, hasil dari kajian ini telah mengenalpasti kesan pelindung dari ARH dalam individu Melayu yang membawa gen HLA-DR7. Kehadiran dominan sel efektor T yang mengekspresi TCR-Vbeta 7 dan 18 mencadangkan peranan gen segmen ini dalam menghasilkan gejala alergi dan menimbulkan persoalan kepentingan interaksi kompleks HLA-TCR dalam penyakit ARH. Keputusan kajian imunofenotip menekankan peranan disregulasi imun dalam mekanisme alergi. Analisis perbandingan dan korelasi mendapati walaupun ujian makmal rutin yang tersebut amat sesuai untuk mendiagnos penyakit, rangsangan secara *in vitro* dan penilaian imunofenotip sel T kawalan boleh dianggap pendekatan yang lebih dipercayai untuk menilai gejala

keterukan penyakit ini (terutama dengan sub-kelas IL-10 dan CD152). Hasil kajian ini tidak menyokong penggunaan ujian diagnostik konvensional dalam menilai gejala keterukan alergi hidung.



ACKNOWLEDGEMENTS

I would like to convey my appreciation to several people who helped make this project and thesis a success. First and foremost, I wish to express my deepest gratitude to my supervisor Associate Prof. Dr. Maha Abdullah, for according me the chance to further my studies to this level and guiding me from the conception of ideas through to the completion of the final written draft. Without her patience, invaluable advices and unconditional support this project would not be completed.

I would like to also extend my sincere appreciation to my co-supervisors, Prof. Dr. Seow Heng Fong, Dr. Yap Yoke Yeow and Associate Prof. Dr. Ranbir Kaulsay who have provided me with their excellent scientific assistance and continuous encouragement through this work.

I wish to also gratefully acknowledge the support of Prof. Dr. Wan Omar Abdullah and Prof. Dr. Abdul Hamid Bin Abdul Rashid whose excellent scientific advices, constructive critics and spiritual guidance have directed me to the right path through the hard days of scientific endeavour.

I would like to also express my gratefulness to the staff and students of the immunology unit for their unconditional assistance and cooperation through my studies. Their valuable contributions will always be treasured.

I also address my sincere thanks to my dear friends Dr. Mirsaed Mirinargesi, Dr. Ehsanollah Ghaznavi and Dr. Amin Yazdani for their sincere assistance and brilliant support.

Finally but certainly not least, I owe my deepest gratitude to my family members; my mother and my brothers for their moral support and encouragement through my studies. My apologies and sincere thanks is addresses to those whom I failed to mention but contributed to this project in any manner.

I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **Peyman Amini** on his Doctor of Philosophy thesis entitled “**Identifying Role of Immune-Dysregulation in Mite-Induced Allergic Rhinitis among Malays**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the degree of Doctor of Philosophy (PhD).

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

PEYMAN AMINI

Date: 17.February.2012

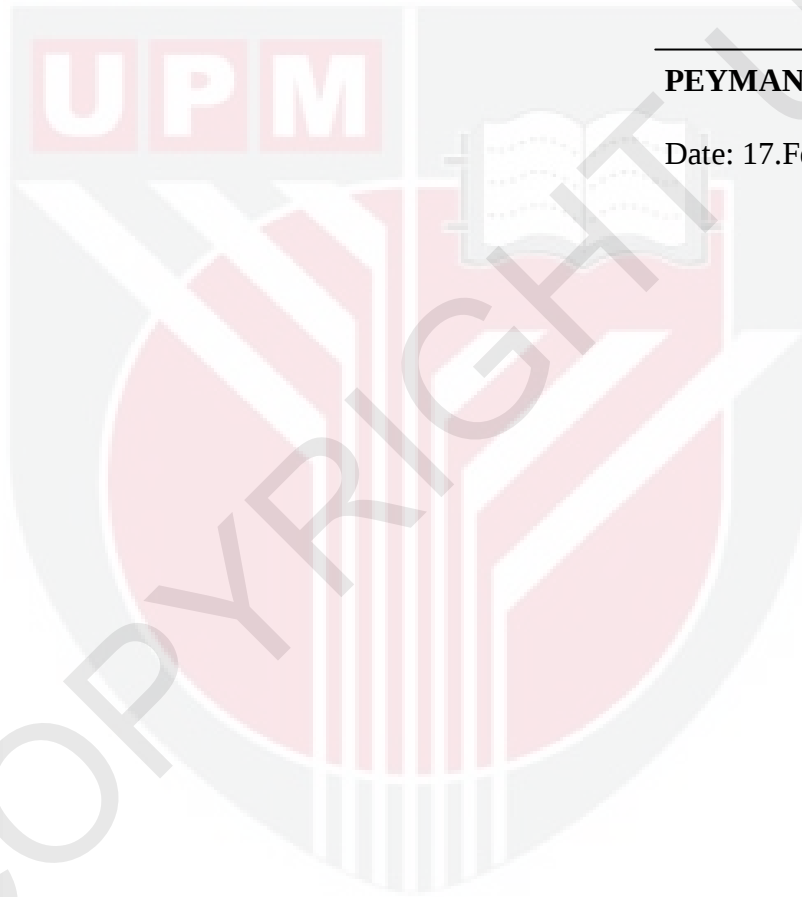


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