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

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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

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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

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

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February 2012

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-Vβ 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-Vβ alleles studied, the expression of Vβ18 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 Vβ7 (*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\(\beta\) 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 Abdulllah PhD

Fakulti: Perubatan Dan Sains Kesihatan

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 merupakan 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
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 segmen TCR 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.\(p\)1 (hama habuk) telah memindahkan penonjolan alel ini kepada yang lain iaitu V\(\beta\)7. Walau bagaimanapun, 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-V\(\beta\) 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. Walau bagaimanapun, setelah sel dikultur dan terutamanya selepas rangsangan khusus dengan hama habuk, didapati peningkatan sel T kawalan untuk kebanyakkan 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-V\(\beta\) 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 mekanisma 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
terukan alergi hidung.
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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).

Members of the Examination Committee are as follows:

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Date:
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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