
Possible association between cockroach allergy and HLA class II antigens

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Background: Susceptibility to the development of allergic diseases is known to be associated with genetic components, as well as environmental factors. Although the genetics of immunoglobulin E, atopy, and asthma are complex, genetic markers are needed to identify populations at risk and to plan intervention studies.

Objective: Human leukocyte antigen (HLA) class II genes play a major role in the control of immune response. We investigated the association between HLA class II alleles of DRB1 and DQB1 and the expression of atopy in cockroach-sensitive patients.

Methods: Levels of total and specific immunoglobulin E were determined. Skin prick tests were performed. HLA class II typing was performed by the Polymerase chain reaction with sequence-specific primers. Distribution of the HLA genotypes of 32 cockroach-positive atopic patients from the inner city were compared with those of 32 healthy, nonatopic controls of Turkish Caucasian origin.

Results: HLA class II gene analysis showed an increase of the HLA-DRB1*0701 and HLA-DQB1*02 alleles in atopic patients compared with nonatopic controls (31.3% vs 3.1% and 50% vs 15.6%, $P_c < 0.036$ and $P_c < 0.021$, respectively). Conversely, HLA-DRB1*15 allele was encountered more frequently in the control subjects. An association between cockroach sensitivity and cutaneous reactivity to other aeroallergens was observed ($P < 0.001$).

Conclusions: It is suggested that the higher frequencies of HLA-DRB1*0701 and HLA-DQB1*02 alleles are probably related to atopy rather than an association between class II antigens and cockroach allergy in this group of polysensitized, atopic individuals. Further studies may lead to a better understanding of the genetically determined susceptibility, and evaluate the individual effects of each locus (or allele) on sensitivity to specific allergens in the Turkish population.

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INTRODUCTION

Cockroach allergens, known as a major component of house dust composition, might contribute significantly to the prevalence of allergic symptoms in predisposed individuals, as the proportion of cockroach-infested houses is increasing.^{1–5} However, exposure to cockroach allergens is not the only risk factor for sensitization, suggesting that genetic factors may play an important role.⁶ Identifying the genes that influence sensitivity to cockroach allergens could lead to a more targeted treatment and, therefore, prevention of atopic sensitization, particularly in the inner-city populations.

It is known that the predisposition to allergic diseases is inherited, even though the mode of inheritance remains unclear. The initial reports on the genetics of atopy and asthma linked to human chromosome 6 genes identified the association of some human leukocyte antigen (HLA) markers by linkage to allergen reactivity.⁷ HLA class II genes are likely candidates for controlling the immune response to common allergens because of their function in the recognition and presentation of intracellularly processed peptides to CD4+ T helper cells.^{8–10} Many studies have tried to identify an association between the expression of atopy to certain allergens

and genetic components, but genetic analysis has been difficult and results have been contradictory.^{11–13} Some authors were unable to confirm a significant link between HLA and atopy as defined by increased total and specific immunoglobulin (Ig)E levels and positive skin prick tests (SPTs), despite the associations between HLA-DR antigens that have been reported for a number of allergens.¹⁴

Various immunogenetic studies have been performed in mite- or pollen-sensitive patients and demonstration of linkage with HLA genes, although there are few data on cockroach allergy. A recently published study on African-Americans found an association between DRB1 and cockroach reactivity.^{15,16} Thus, the aim of this study was to determine whether an increased prevalence of sensitization to cockroach allergens was associated with HLA class II genes in a group of patients with asthma and/or rhinitis living in an urban area, ie, Ankara, the capital of Turkey.

METHODS

We evaluated the immunogenetics of cockroach allergen sensitivity in two groups of unrelated adults.

The study population comprised patients who were referred to our allergy department with a clinical history of allergic disorders of the respiratory system. After giving informed consent, the subjects were asked to complete a questionnaire including age, sex, atopic background, and duration of symptoms. Allergy diagnosis was confirmed by

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history, physical examination, and diagnostic tests. The protocol was approved by the ethics committee.

SPTs were performed with the commercially available cockroach antigen *Blattella germanica* along with a common battery of standardized inhalant allergen extracts (Stallergènes SA, Pasteur, France). Histamine dihydrochloride 10 mg/mL and phenolated glycerol-saline served as positive and negative controls and a mean wheal diameter of 3 mm or greater than control was considered positive. Serum samples were collected from each subject for determination of total IgE (Abbott Laboratories, Abbott Park, IL). CLA-TOP System (Turkish Panel 36, MAST Immunsystems, Mountain View, CA) was used to measure specific IgE levels. Cockroach sensitivity was defined as a positive SPT and/or detectable specific IgE to *B. germanica*.

Genomic DNA was extracted from peripheral blood lymphocytes by phenol-chloroform extraction and ethanol precipitation accordingly. Polymerase chain reaction with sequence-specific primers (One Lambda, Los Angeles, CA) was used for PCR amplification. Oligotyping was performed to find the frequency of HLA class II alleles. The amplification of 19 specific alleles were carried out according to the manufacturer's instructions and alleles were evaluated with regard to HLA-DRB1 and DQB1 loci.

Statistics

DRB1 and DQB1 allele frequencies were determined by direct counting. Frequencies of each allele were compared using the χ^2 test. A *P* value <0.05 was considered significant, and the *P* value was corrected for the number of alleles tested. The Statistical Package for Social Sciences (SPSS, 9.05, Chicago, IL) for Windows was used to analyze the data.

RESULTS

A total of 32 cockroach allergic/atopic patients were enrolled in this study (Table 1). In cockroach-sensitive patients, 65.5% were women. Of the 32 patients tested, 50% claimed rhinitis, followed by asthmatic symptoms, with the duration of 7.4 ± 5 years. Most patients were polysensitized, having positive SPTs mainly regarding pollens (43%) and mite allergens

(40%), whereas only nine subjects showed isolated cockroach positivity. The control group consisted of 32 healthy transplant (kidney or liver) donor individuals (20 male and 12 female, mean age of 42.2 ± 8.3 years) previously typed in our laboratory. SPTs were performed on control population and all were negative.

The distribution of DRB1 and DQB1 alleles in cockroach-positive polysensitized patients and the control subjects are shown in Tables 2 and 3. The frequency of HLA-DRB1*0701 and HLA-DQB1*02 were found to be significantly higher in atopics compared with the nonatopic controls (31.3% vs 3.1% and 50% vs 15.6%, *P* < 0.036 and *P* < 0.021, respectively). By contrast, the frequency of HLA-DRB1*15 allele was increased in the control group (28.1% vs 3.1%); however, this difference did not reach statistical significance when the *P* value was corrected for multiple comparisons.

DISCUSSION

Over the past 10 years, a tremendous interest has grown in identifying the genetics of allergic diseases, even though the mode of inheritance remains unclear. Cockroaches commonly found in urban dwellings worldwide have increasingly been recognized as one of the major causes of inhalant allergy and asthma in Turkey as well.¹⁻³ As genetically determined susceptibility may be responsible for a significant influence on the regulation of type I hypersensitivity, an association between cockroach-specific IgE immune response and HLA class II genes were studied.

In this study, we analyzed the immunogenetics of sensitization to *B. germanica*, the German cockroach that has been regarded as the most widespread with a high immunologic potential.^{1,2} We found that the frequencies of the HLA class II alleles DRB1*0701 and DQB1*02 were higher in cockroach-positive atopic patients. The frequency of DRB1*15 was increased in nonatopic healthy controls, but its distribution did not reach statistical significance after *P* value correction.

To our knowledge, this is the first report from Europe of an HLA association with susceptibility to cockroach allergens. Donfack et al¹⁵ hypothesized that the primary association

Table 1. The Characteristics of Cockroach-Positive Atopic Patients

	n = 32		
	F, 21 (65.5%)	M, 11 (34.4%)	Mean
Age (years)*	44 ± 5.5 (17–47)	36 ± 1.4 (23–57)	42 ± 2.5 (17–57)
T.IgE (IU/mL)*	491.4 ± 51.4 (19.8–2,431)	492.5 ± 58.6 (38.5–1,787.8)	491.8 ± 51.4 (19.8–2,431)
Cockroach Spe IgE+	13 (40.6%)	6 (18.8%)	19 (59.4%)
Cockroach SPT+	11 (34.4%)	8 (25%)	19 (59.4%)
Asthma	3 (9.4%)	3 (9.4%)	6 (18.8%)
Rhinitis	10 (31.3%)	6 (18.8%)	16 (50%)
Asthma + rhinitis	8 (25%)	2 (6.3%)	10 (31.3%)
Duration of symptoms (years)*	7.5 ± 5.7	7.1 ± 5.6	7.4 ± 5

* Mean ± SD values.

Table 2. Distribution of HLA-DRB1 Alleles among Cockroach-Positive Atopic Patients and Control Group

Alleles	Patients group		Control group		P	Pc
	(n = 32)		(n = 32)			
	n	%	n	%		
DR1	3	9.4	1	3.1	NS	NS
DR3	6	18.8	4	12.5	NS	NS
DR4	8	25	7	21.9	NS	NS
DR7	10	31.3	1	3.1	0.003	0.036
DR8	1	3.1	1	3.1	NS	NS
DR9	1	3.1	0	0	NS	NS
DR11	13	40.7	18	56.3	NS	NS
DR12	3	9.4	1	3.1	NS	NS
DR13	4	12.5	6	18.8	NS	NS
DR14	2	6.3	2	6.3	NS	NS
DR15	1	3.1	9	28.1	0.006	NS
DR16	3	9.4	3	9.4	NS	NS

NS, Not significant; Pc, corrected P values.

Table 3. Distribution of HLA-DQB1 Alleles among Cockroach-Positive Atopic Patients and Control Group

Alleles	Patients group		Control group		P	Pc
	(n = 32)		(n = 32)			
	n	%	n	%		
DQ2	16	50	5	15.6	0.003	0.021
DQ4	0	0	2	6.3	NS	NS
DQ5	8	25	6	18.8	NS	NS
DQ6	6	18.8	15	46.9	NS	NS
DQ7	16	50	19	59.4	NS	NS
DQ8	7	21.9	6	18.8	NS	NS
DQ9	1	3.1	0	0	NS	NS

NS, Not significant; Pc, corrected P values.

with cockroach allergies was with DRB1*01 alleles, DRB1*0101 in the Hutterites and DRB1*0102 in the African-Americans studied. However, comparisons between studies are difficult because of differences in ethnic groups and in the phenotypic definitions chosen for allergy and atopy in each particular study.^{11,15-17} The differences between the samples may reflect differences in DRB1 susceptibility alleles in each population, and the random sampling of individuals may be inadequate for association studies of genetic factors and specific IgE immune response. Further, HLA-DR7 and HLA-DQ2 are in strong *linkage disequilibrium*, and the concomitant association with specific sensitivity may only be a reflection of this phenomenon. This is of fundamental importance to ascertain which is the real "causative" locus.

Although the frequencies of these two alleles at HLA loci were higher in cockroach-positive polysensitized patients, there is not an association with cockroach allergy attributable to a lack of atopic controls. In contrast, similar data were

reproduced in the preliminary results of an investigation concerning all atopic patients.¹⁸ In this ongoing study, we found the same positive associations with HLA class II alleles, parallel to the present study. As identical data were observed, the results of our study may not only be specific to cockroach sensitivity, but also to atopy in the Turkish population. This hypothesis was also supported by the work of Aron et al.¹⁹ They observed that HLA-DR4 and -DR7 alleles were strong risk factors for atopy, in unrelated Caucasian patients. Therefore, we could assume that an increased frequency for DRB7 and DQB2 alleles was more significantly related to atopy rather than to specific sensitivity.

CONCLUSION

It is difficult to determine an association between HLA alleles and specific IgE response to cockroach allergens in the Turkish population with this group of polysensitized, atopic individuals. As the conclusive results of better controlled studies within families from different ethnic origins will be reproduced, the more complex pathways involved in the pathogenesis of atopic sensitization can be identified.

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