

Short communication

Pocket 4 in the HLA-DRB1 antigen-binding groove: an association with atopy

Background: Many studies have attempted to identify an association between HLA genes and atopy, given the role of HLA molecules in the regulation of the immune response. In the case of house-dust mites, it is difficult to find an association with a particular HLA allele, due to the complexity of the allergen. The objective was to investigate whether HLA-DRB1 functional groups are better correlated with the atopic disease in our population than DRB1 alleles.

Methods: The method was reanalysis of the HLA-DRB1 data of a previous case/control study.

Results: The “Dr” group was found to be associated with the atopic disease in our population.

Conclusions: Grouping HLA-DRB1 alleles into functional categories may assist in the search for predictive factors in relation to atopic disease.

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HLA-DRB1 is an HLA (human leukocyte antigen) class II gene that encodes the β chain of HLA-DR molecules. These are cell-surface glycoproteins involved in the recognition and presentation of exogenous peptides to T cells. Atopy is a multifactorial condition, depending upon both genetic and environmental influences, and characterized by persistent IgE responses to ubiquitous aeroallergens. After initial sensitization in predisposed individuals, re-exposure to the allergen results in the IgE-mediated degranulation of mast cells, inflammation of airways, wheezing, and rhinitis.

Rhinoconjunctivitis and bronchial asthma are atopic diseases with a high prevalence in the Canary Islands (Spain): 43.1% of the young population suffer from allergic respiratory diseases, according to the study of García-Ramos et al. (1). The geographic situation of the archipelago, a subtropical area at sea level, provides adequate climatic conditions (warm temperatures and a high relative humidity) for the growth of mites: in fact,

the house-dust mite *Dermatophagoides pteronyssinus* (Der p) is the most prevalent allergen (1–3).

Given that the incidence, prevalence, and severity of atopic diseases are increasing in the industrialized countries (4), there is much interest in prevention. In the case of house-dust mites, epidemiologic studies have shown the relationship between the level of exposure and the sensitization to the allergen or the presence of respiratory symptoms (5–7). Early detection of susceptible subjects would allow the application of preventive measures.

Many studies have attempted to identify an association between HLA genes and atopy, given the role of HLA molecules in the regulation of the immune response. Several associations between HLA-DRB1 alleles and atopic disease have been described (8–10). A crucial element in these studies was the use of well-defined, relatively simple, and extremely purified antigens, such as certain pollen-derived allergens.

In the case of house-dust mites, the epidemiologic studies have not always found an association with a particular HLA allele (11–14). We have previously reported the existence of genetic linkage between the HLA region and the response to Der p in our population, together with a lack of association with HLA-DRB1, -DQB1, and -DQA1 alleles (15). It is clear that Der p-derived allergens contain several overlapping T-cell epitopes restricted by different HLA-class II molecules (16, 17). This phenomenon limits the possibility of finding an association between particular HLA alleles and the response to the allergen.

Recent studies of the structure of HLA-DR molecules have identified subregions (pockets) in the DR binding groove which exert a major influence on the binding of the peptide, and its subsequent recognition by T cells (18). Particularly, the polymorphic residues 70, 71, and 74 of the β chain, located in pocket 4, play a critical role in the recognition of the HLA-DR/peptide complex by the CD4⁺ helper T cell (19). It is possible to identify seven groups of HLA-DRB1 alleles on the basis of the physicochemical characteristics, charge in particular, of the polymorphic residues (Table 1). These functional groups appear to be associated with susceptibility or resistance to particular autoimmune and infectious diseases (such as rheumatoid arthritis, allergic bronchopulmonary aspergillosis, and type 1 diabetes), thus explaining the apparently unlike results of many studies (20).

Grouping HLA-DRB1 alleles into functional categories may assist in understanding the mechanistic basis of atopy, resolving current paradoxes in the association of HLA with the disease. On the basis of this new approach, we have reanalyzed the HLA-DRB1 data of our previous case/control study, which was performed with a carefully selected group of atopic patients and nonatopic control subjects.

Material and methods

A total of 161 unrelated individuals from the island of Gran Canaria (Canary Islands, Spain) were recruited for the study by the physicians at the allergy section of

our hospital. Subjects were diagnosed as atopic if they met both of the two following criteria:

- 1) diagnosis of rhinoconjunctivitis and/or bronchial asthma
- 2) positive skin prick test to Der p.

Subjects negative to both criteria were classified as nonatopic. Informed consent was obtained from the subjects participating in the study. The protocol was approved by the investigation and ethics committee of our hospital.

The diagnosis of rhinoconjunctivitis and bronchial asthma was assessed by the clinical history and physical examination of the subjects, according to international criteria.

Skin prick tests were carried out in patients and control subjects by a previously described standard method (15), with a panel of 18 common allergens including mites, molds, pollens, and animal danders. A wheal with a diameter 3 mm greater than the saline control was considered a positive response.

Genomic DNA from each subject was isolated from a whole-blood sample by phenol/chloroform extraction and ethanol precipitation, by a standard procedure (15).

HLA-DRB1 typing was performed by the polymerase chain reaction with sequence-specific primers (PCR-SSP), by a previously described method (15). Frequencies of HLA-DRB1 groups were compared by Fisher's exact test. The *P* values were corrected by multiplying them by the number of alleles tested. A *P* value of less than 0.05 was considered to indicate a statistically significant difference between groups.

Results

Our population consisted of 76 atopic and 85 nonatopic individuals. Out of these 161 subjects, 53 were male (46% atopic) and 108 were female (48% atopic). The mean age was 36.7 ± 13.6 (SD) years. Mean ages, standard deviations, and ranges were almost identical for the two sexes.

The results of the study of the possible association of atopy with DRB1 functional groups are shown in Table 2. Our analysis showed an association of the "Dr" group with the atopic disease in our population.

Discussion

Atopy is a multifactorial condition, depending upon both genetic and environmental influences. Despite considerable evidence of heritability, it has been very difficult to determine genetic markers that allow the identification of predisposed individuals, due to the genetic heterogeneity of the disease and its variable expression.

Table 1. HLA-DRB1 functional groups, defined by residues β 70, β 71, and β 74

Group	Residues [§]	Disease association [†]
A	QRA, QKA, RRA, QRL	Rheumatoid arthritis
De	DEA	Pemphigus vulgaris
Dr	DRA, DRL, DRV, DKA	Allergic bronchopulmonary aspergillosis
E	QRE, RRE	Hashimoto's thyroiditis
Q	DRQ	Psoriasis
R	QKR	Systemic lupus erythematosus
a	QAA	Multiple sclerosis

[§] Positions 70/71/74. Q: glutamine; R: arginine; A: alanine; K: lysine; L: leucine; D: aspartic acid; E: glutamic acid; V: valine.

[†] Ref. 20.

Table 2. Allelic frequencies (in percentages) of HLA-DRB1 functional groups

Group	Patients (N)	Controls (N)	P	P _c	OR	CI
A	17.1 (26)	24.7 (42)	0.03	NS		
De	25.0 (38)	23.6 (40)	0.10	NS		
Dr	27.6 (42)	13.5 (23)	0.0008	0.005	2.44	1.39–4.29
E	1.3 (2)	4.1 (7)	0.09	NS		
Q	13.2 (20)	14.7 (25)	0.12	NS		
R	12.5 (19)	9.4 (16)	0.10	NS		
a	3.3 (5)	10.0 (17)	0.01	NS		
Total	100 (152)	100 (170)				

N: chromosomes; P_c: corrected P value; NS: not significant; OR: odds ratio; CI: confidence interval.

Given the key role of HLA molecules in the regulation of the immune response, genetic epidemiologic studies have attempted to relate responsiveness to specific allergens to HLA type. A former study by our group did not find any evidence of association between allergy to Der p and particular HLA-DRB1/DQB1/DQA1 alleles (15). The exposure to a broad variety of Der p-derived peptides makes it difficult to define the contribution of the HLA-class II genes to the atopic disease: previous studies have corroborated the difficulty of establishing particular associations between allergy to house-dust mite and HLA class II alleles (11–13).

In this new analysis of the association between HLA-DRB1 gene and atopy in our population, we have focused on the DRB1 functional groups. This recently described approach seems to explain apparent contradictions in the results of similar studies (20), relative to autoimmune and infectious diseases, and has not been applied to the study of atopy yet. We have obtained a significant result: a positive association of the “Dr” group with the atopic disease in our population. Table 2 shows the allelic frequencies, but when we count individuals instead of chromosomes, the “Dr” group is found in 44% of patients, an outstanding finding given the complexity of the antigen and the use of Der p crude extract in prick tests.

Our results suggest that subjects carrying a “Dr” DRB1 allele are more predisposed to develop atopic disease. This hypothesis is strengthened by the results of

an additional association study in which we have investigated the genotypes formed by all the possible group combinations. We found that the “Dr” homozygotic individuals seem to be much more susceptible to the disease (OR = 5.57, CI = 1.16–26.68, P = 0.01). In this case, the P value becomes nonsignificant after correction, perhaps because of the small figures in each category (nine patients and two control subjects, but among the 28 possible genotypes, the homozygotic “Dr” was the only one that showed statistical significance before correction).

So far, nucleotide sequence polymorphisms have identified more than 200 distinct HLA-DRB1 alleles, but nucleotide differences do not account well for the observed variations in associations with disease susceptibility or resistance. Differences in the amino-acid sequence are much more important, since a single substitution at a critical residue of the HLA binding groove can alter the ability to bind antigenic peptides, totally changing the sign of the immune response. The charged residues located in pocket 4, among others, seem to be critical in defining the selectivity of peptide binding to DR molecules, as confirmed by site-directed mutagenesis (20).

In our population, the HLA-DR functional groups have proved to be more useful than DRB1 alleles in the search for an HLA–atopy association. These groups are determined by polymorphism of residues β70, β71, and β74, located in pocket 4 of the DR binding groove, although there are other polymorphic residues which also influence the allergen recognition by T cells. This functional approach could be a better strategy in association studies of complex antigens, together with a more accurate definition of the individual’s responsiveness to the allergen.

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