

Association of Responsiveness to the Major Pollen Allergen of *Parietaria officinalis* with HLA-DRB1* Alleles

A Multicenter Study

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ABSTRACT: *Parietaria*, a plant belonging to the family of *Urticaceae*, is a major source of allergenic pollen in Europe. In the context of a multinational study, we investigated whether in allergic subjects antibody response towards Par o 1, the major allergen from *P. officinalis*, was associated with defined HLA-DRB1* alleles.

The study population consisted of 234 allergic patients: 65 from Bulgaria, 30 from Israel, 99 from Italy, and 40 from Spain.

In the Italian study group, the prevalence of ST positivity to *Parietaria* was 77%. In *Parietaria* ST-positive subjects, the prevalences of IgG and IgE serum Ab towards Par o 1 were 91% and 75%, respectively. HLA-DRB1*1101 and/or 1104 were significantly positively associated with the presence of IgG Ab and with high levels of IgE Ab towards this allergen ($p = 0.0007$ and $p = 0.012$, respectively).

In the Spanish study group, the positive association of DR1100 with responsiveness to Par o 1 was confirmed ($p = 0.02$, RR = 4, and $p = 0.002$, RR = 7, for IgG and IgE Ab, respectively). None of the Bulgarian patients had IgE Ab to Par o 1, whereas IgG Ab response was observed in 7 out of 65 subjects and was positively associated with DRB1*1101 and/or 1104 ($p = 0.025$). In the Israeli study group, responsiveness to Par o 1 was not associated with specific HLA-DRB1* alleles.

In conclusion, this study shows that in allergic patients from three European populations antibody response to the major allergen from the pollen of *Parietaria* is associated with HLA-DRB1* 1101 and/or 1104. Our data suggest that this association is stronger in subjects monosensitized to *Parietaria*. *Human Immunology* 46, 100–106 (1996)

ABBREVIATIONS

BBS borate-buffered saline
CIE crossed immunoelectrophoresis
DARIA double antibody radioimmunoassay
MHC major histocompatibility complex

PCR polymerase chain reaction
SDS-PAGE sodium dodecylsulfate–polyacrylamide gel electrophoresis
SSO sequence-specific oligonucleotide
ST skin test

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INTRODUCTION

The concept that alleles of the major histocompatibility complex (MHC) (HLA in humans) control specific immune responsiveness was introduced by Mc Devitt and Tyan [1], who showed that, in inbred mice, the ability to produce antibody (Ab) response to certain antigens is inherited as a dominant trait and cosegregates with defined class II alleles. In humans, one of the first unequivocal examples of this phenomenon is represented by the association of responsiveness to a minor *Ambrosia* allergen, Amb a 5, with HLA-DR2 [2–4]. Epidemiologic surveys have documented the association of defined HLA-DRB or -DQ alleles with responsiveness/nonresponsiveness towards several other allergens [5–12]. More recently, a family-based study on several specific allergies indicated that responsiveness to the mold allergen Alt a 1 and to the cat allergen Fel d 1 are genetically linked with HLA-DR4 and -DR1, respectively [13]. The present report describes the results of a multinational study [14] designed to investigate whether, in allergic subjects, allergy to *Parietaria* and responsiveness to the major allergen Par o 1 were associated with specific HLA-DRB1* alleles.

Parietaria (pellitory of the wall), a weed of the family of *Urticaceae* (nettle), is a major source of allergenic pollen in Mediterranean Europe. The prevalence of *Parietaria*-specific allergy among adults reaches 70%–80% in several regions of Italy and ranges between 20% and 60% in Spain [15]. The epidemiologically and clinically most relevant species are *P. officinalis* and *P. judaica*. In Italy these species occupy partially overlapping areas; *P. judaica* is reported to be more prevalent in Spain (with *P. lusitanica*) and in Israel (with *P. mauritanica*) and *P. officinalis* is more prevalent in Bulgaria [15, 16]. The pollens from *P. officinalis* and *P. judaica* contain highly cross-reactive allergens, and extracts from these species can be used interchangeably in diagnosis and therapy [17, 18]. The major allergens Par j 1 and Par o 1, glycoproteins with a mw of 12–14 kd, are extensively cross-reactive and have highly homologous N-terminal amino acid sequences [19–21].

MATERIALS AND METHODS

Study population. The study group consisted of 234 unrelated allergic patients, aged 15–54 years: 99 were from Italy, 65 from Bulgaria, 40 from Spain, and 30 from Israel. Patients were informed volunteers and had not received immunotherapy with *Parietaria* extracts. They completed a standard questionnaire which included information on clinical history and allergen exposure. Patients were skin positive by prick test (wheal diameter

3 mm larger than the negative control) towards at least one extract from a panel of 18 glycerinated extracts of common inhalant allergens (Hollister-Stier Laboratories) and/or had IgE Ab to at least one of the following allergens: Alt a 1, Amb a 1, Amb a 5, Bet v 1, Cry j 1, Der p 1, Fel d 1, Lol p 1, Lol p 5, Ole e 1, Par o 1, or Poa p 9 [14].

All patients were skin prick tested with *P. judaica* and *P. officinalis* pollen extracts; as results were always concordant between these species, they will be indicated here as *Parietaria* skin test (ST) positive/negative. Spanish patients had been selected for allergy to olive.

Sera and cells. Serum obtained from each patient was aliquoted and stored at -70°C . White blood cells were obtained by centrifugation from 5 ml of blood in 0.12 ml of 0.4 M EDTA (pH 7.4) after addition of 5 ml of phytohemagglutinin-P (PHA-P) (Difco). The pellet was resuspended in phosphate-buffered saline (PBS), pH 7.5, at approximately 250,000 cells/ml and 1-ml aliquots were stored at -70°C .

Allergens. *P. judaica* and *P. officinalis* pollens were purchased from Allergon AB (Sweden). Par o 1 was purified from the pollen extract by Sephadex G75 and G50 gel filtration and DE52 ion exchange chromatography [21]. From 1 g of pollen 500 μg of allergen were recovered. In sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) this preparation was 95% homogeneous. Par j 1 was purified using essentially the same procedure.

Radiolabeling. Purified allergen, 40 μg , was labeled using 2 mCi of ^{125}I (3.7 GBq/ml, Amersham, UK) by the chloramine T procedure [22]. Aliquots of 100 μl of a solution containing 1 $\mu\text{g}/\text{ml}$ (10–20 106 cpm/ml) of radiolabeled protein in borate-buffered saline (BBS) pH 8.4 containing 5% bovine serum albumin (BSA), were stored at -20°C .

Double antibody radioimmunoassay (DARIA). The assay was essentially performed as already described [23]. For each serum, the levels of IgG and IgE Ab towards Par o 1 were determined in parallel. For IgG Ab, 100 μl of 1/10 serum in BBS or of further dilutions in IgG carrier (1/10 control human serum), were incubated for 4 hours at room temperature with 100 μl of BBS containing 1 ng of radiolabeled Par o 1 (10,000–20,000 cpm) in 100 μl of BBS. Antigen–antibody complexes were precipitated by adding the appropriate amount of goat anti-human IgG for 16 hours at 4°C . For IgE Ab, 50 μl of serum were mixed with 50 μl of the appropriate dilution of an IgE myeloma protein, prior to addition of the radiolabeled

allergen. Antigen-antibody complexes were precipitated by adding the appropriate amount of goat anti-human IgE. Precipitates were collected on Whatman GF/A glass fiber paper filters. After washing (with BBS) the radioactivity retained on filters was measured by a gamma counter. Each experiment included a reference dose-response curve obtained with twofold serial dilutions (1/10 to 1/20,480) of a serum having a very high level of IgG Ab towards Par o 1 and a negative control (a serum with undetectable amount of Ab towards Par o 1). Par o 1 binding was considered statistically significant if cpm bound by the tested sample were more than 1.3-fold the cpm bound by the negative control. IgG and IgE Ab levels were expressed as U/ml, by extrapolation from the reference curve, according to the formula: $(a - b)/(B_{\max} - b)$, where b = background cpm (bound by negative control), a = cpm bound by tested serum, and $B_{\max}$ = cpm bound by the reference serum at 1/10 dilution.

Experiments were performed in duplicate and results expressed as means. Determinations were repeated if the difference between duplicates was more than 11%. Background cpm ranged between 2.5% and 3.3% and 1.9% and 2.5% of $B_{\max}$ values for IgG and IgE DARIA, respectively.

Crossed immunoelectrophoresis. Crossed immunoelectrophoresis (CIE) was performed according to established protocols as described elsewhere [21].

Total IgE. Total serum IgE level was determined by radioimmunoassay (Pharmacia CAP system; Pharmacia, Upsala, Sweden), following the manufacturer's instructions. The measuring range was 2–2000 kU/l.

HLA-DRB1* typing. HLA-DRB1* typing of Bulgarian, Israeli, and Spanish patients was performed using sequence-specific oligonucleotides (SSOs) (11th Histocompatibility Workshop) and dot-blot analysis [8, 9]. In the Spanish patients, DRB1*1100 subtypes (1101/02/03/04) were not discriminated.

DRB1* typing of Italian patients was achieved combining dot-blot and DNA heteroduplex analysis [24, 25]. Several cell lines (most of which were from the Tenth Histocompatibility Workshop [26] were used as negative and/or positive controls in every step of the entire procedure. Samples were first subdivided into DRB1*01, DRB1*15/16, DRB1*04, DRB1*07, DRB1*09, DRB1*10, and DR52-DRB1 associated (DRB1*03, DRB1*11, DRB1*12, DRB1*13, DRB1*14 and DRB1*08) groups of alleles following the 11th Histocompatibility Workshop DRB1* typing procedure, foreseeing an amplification with DRB1 generic primers (DRBAMP-A and DRBAMP-B) and subsequent hybridizations with a series of SSOs [24, 27]. DRB1*01, DRB1*15/16-, and DR52-DRB1*-associated group-

positive individuals were then subjected to a second round of amplification for further characterization. The 5'-end specific primers DRBAMP-1, DRBAMP-2, and DRBAMP-3 in combination with DRBAMP-B were used for the DRB1*01-, DRB1*15/16-, and DR52-DRB1*-associated group, respectively [27]. DRB1*15/16 alleles were distinguished following a previously described published procedure [28], whereas DRB1*01 and DR52-DRB1* alleles were distinguished by hybridizing the samples separately with selected SSO probes selected from the 11th Workshop panel [28].

DR52-DRB1* data were refined, in some cases, by group-specific polymerase chain reaction (PCR) and DNA heteroduplex analysis by an adaptation of the procedure reported in D'Amato and Sorrentino [25].

Statistic analyses. The associations were investigated by nonparametric (χ^2) and parametric analysis (stepwise multiple regression). In multiple regression analyses the independent variables were each of the different HLA-DRB1* alleles, log (total IgE), and each patient age and gender [3]. Unless otherwise indicated, p is referred to χ^2 analysis (Yates-corrected, but uncorrected for multiple comparisons).

RESULTS

Prevalence of Skin Test Positivity to *Parietaria* and of IgE/IgG Antibody Response to Par o 1

The prevalences of ST positivity to *Parietaria* pollen extract in the Italian, Spanish, Israeli, and Bulgarian study groups were 77%, 33%, 27%, and 42%, respectively (Table 1). The proportions of *Parietaria* ST-positive patients who had IgG and IgE antibody towards Par o 1 (IgG and IgE Par o 1 responders) were 91% and 73% in the Italians, 92% and 67% in the Spanish, and 88% and 75% in the Israeli, respectively. None of the Bulgarian patients had detectable levels of IgE Ab and seven had IgG Ab towards Par o 1 (Table 1).

The Italian study group, which included the largest number of patients and where allergy to *Parietaria* was more prevalent, will be considered more in detail. Fifty-six subjects had IgG and IgE Ab towards Par o 1, whereas 19 had only IgG Ab towards this allergen. Seventy-seven patients were ST positive to *Parietaria*. Twenty-nine (38%) of *Parietaria* ST-positive patients were ST negative towards the other allergenic extracts tested (*Parietaria* monosensitized), whereas the remaining subjects had multiple sensitizations (multisensitized).

HLA-DRB1* Association

Italian patients. The frequency of selected HLA-DRB1* alleles in Par o 1 responders and nonresponders is reported in Table 2.

TABLE 1 Study group

	No. of Patients			
	Italian	Bulgarian	Spanish	Israeli
Total	99	65	40	30
SR positive to <i>Parietaria</i>	77 (77%)	27 (42%)	13 (32%)	8 (27%)
With IgG Ab to Par o 1	75 (76%)	7 (11%)	11 (28%)	7 (23%)
With IgE Ab to Par o 1	56 (56%)	0	8 (20%)	6 (20%)
Level of IgG Ab to Par o 1 ^a	591 $\pm$ 593 U/ml	115 $\pm$ 40 U/ml	<740 $\pm$ 119 U/ml	330 $\pm$ 141 U/ml
Level of IgE Ab to Par o 1 ^a	229 $\pm$ 245 U/ml		182 $\pm$ 143 U/ml	254 $\pm$ 178 U/ml

^aArithmetic means of IgE and IgG Ab levels expressed in arbitrary units calculated as described in *Materials and Methods*.

In total, 68 patients were HLA-DRB1*1100. These were subdivided, according to our typing procedure, into two groups: DRB1*1101 and/or 04 and DRB1*1102 and/or 03. The frequency of DRB1*1101 and/or 04 was significantly increased with respect to the expected prevalence in the Italian population (69% as compared to 39% [11th International HLA Workshop. Data Analysis Book II Addendum] $p < 0.003$).

In *Parietaria* ST-positive patients, the proportion of DRB1*1101 and/or 04 was increased (66%) as compared to *Parietaria* ST-negative subjects (55%). In subjects monosensitized to *Parietaria* this proportion was 76% (RR = 1.9).

A significant excess of DRB1*1101 and/or 04 was observed in IgG Ab responders as compared to nonresponders ($p = 0.0007$; Table 2). The proportion of these alleles was increased in *Parietaria* monosensitized patients as compared to multisensitized patients (78% and 67%, respectively) and in patients (19 subjects) who had

IgG but not IgE Ab towards Par o 1 as compared to those (56 subjects) who had Ab of both isotypes (79% and 71%, respectively). All patients (6 subjects) who had only IgG Ab response towards Par o 1 and were ST negative to *Parietaria* were DRB1*1101 and/or 04.

The difference in the frequency of DRB1*1101 and/or 04 between IgE Ab responders and nonresponders to Par o 1 was smaller but still significant by regression analysis ($p = 0.012$; Table 2). The proportion of DRB1*1101 and/or 04 was increased in Par o 1 monosensitized patients as compared to multisensitized Par o 1 responders (81% and 66%, respectively).

The frequency of DRB1*03 was significantly decreased in Par o 1 responders as compared to nonresponders, where it was similar to that of the general population (20%; 11th International HLA Workshop: Data Analysis Book II Addendum). The negative association was significant by χ^2 and multiple regression analysis (Table 2).

TABLE 2 Italian patients: prevalence of HLA-DRB1* alleles in responders and nonresponders to Par o 1

DRB1*	No. of patients with allele (%)		$p1^a$		$p2^b$		No. of patients with allele (%)		$p1^a$	$p2^b$
	With IgG Ab to Par o 1 (N = 75)	Without IgG Ab to Par o 1 (N = 24)					With IgE Ab to Par o 1 (N = 56)	Without IgE Ab to Par o 1 (N = 43)		
01	9 (13)	3 (13)	ns	ns			7 (13)	5 (12)	ns	ns
1500	8 (11)	3 (13)	ns	ns			8 (14)	3 (7)	ns	ns
1600	5 (7)	1 (4)	ns	ns			3 (5)	7 (13)	ns	ns
03	4 (5)	4 (17)	ns	ns			1 (2)	7 (16)	0.024(-)	0.012(-)
04	8 (11)	1 (4)	ns	ns			7 (13)	2 (5)	ns	ns
07	10 (13)	5 (21)	ns	ns			7 (13)	8 (19)	ns	ns
08	3 (4)	2 (8)	ns	ns			3 (5)	2 (5)	ns	ns
1101/04	55 (73)	8 (30)	0.0007	0.003			40 (71)	23 (54)	ns	0.012
1102/03	2 (3)	3 (13)	ns	ns			1 (2)	4 (9)	ns	ns
1300	18 (24)	5 (21)	ns	ns			16 (29)	7 (16)	ns	ns
14	5 (7)	5 (20)	ns	ns			4 (7)	6 (14)	ns	ns

^a $p1$, χ^2 analysis.

^b $p2$, multiple regression analysis.

ns, not significant.

Spanish patients. Of the 40 Spanish patients, 11 had IgG Ab and 8 IgE Ab towards Par o 1 (Table 1). Five subjects (13%) were DRB1*1100. DRB1*1100 was significantly positively associated with IgG and IgE Ab response towards Par o 1 (Table 3) and with ST positivity to *Parietaria* ($p = 0.05$, RR = 3.1). IgG Ab response was positively associated also with DRB1*0 (Table 3). The frequency of DRB1*03 did not significantly differ between responders and nonresponders. There were 14 DRB1*03 patients of which five responded to Par o 1 with IgG and four with IgE Ab. Three of the five IgG Ab responders and three of four IgE Ab responders were also DRB1*1100 and the remaining two responders were also DRB1*01, whereas none of the nine DRB1*03 non-responders had these alleles.

Bulgarian patients. None of the 65 Bulgarian patients had detectable levels of IgE Ab towards Par o 1, but seven had a low level of IgG Ab towards this allergen (Table 1). Eighteen subjects (28%) were DRB1*1101 and/or 04. Five of the responders had these alleles ($p = 0.025$, RR = 3.2).

Israeli patients. Of the 30 Israeli patients, eight had IgG Ab and seven had IgE Ab towards Par o 1 (Table 1). Eleven subjects (37%) were HLA-DRB1*1101 and/or 04. A comparison of allele frequency between responders and nonresponders to Par o 1 did not reveal significant biases of distribution.

When all data from patients of different nationalities were combined, the cumulative relative risk for IgG and IgE Ab response towards Par o 1 associated with HLA-DRB1*1100 (01,02,03,04) were 2.6 and 2.4, respectively ($p < 0.0001$ and 0.0002 for IgG and IgE Ab, respectively).

DISCUSSION

In the Italian study group, parametric and nonparametric analyses documented a statistically significant positive association of DRB1*1101 and/or 1104 with the presence of IgG and IgE Ab towards Par o 1 (Table 2). This observation confirms a previous study from our group (in a population unrelated to that of the present report), indicating that the HLA specificity DR5 was positively associated with IgE Ab response to Par o 1 [29]. The association was confirmed in the Spanish patients (Table 3). DRB1*1101 and/or 04 were significantly associated with IgG Ab towards Par o 1 also in the Bulgarians patients (none of which had IgE Ab towards Par o 1). In contrast, in the Israeli study group no specific allele was associated with responsiveness to Par o 1. A possible explanation is that these patients were exposed to species or varieties of *Parietaria* different from those prevalent in Europe.

In the Italian study group, HLA-DRB1*03 was significantly negatively associated with IgE Ab response to Par o 1 (Table 2) and with a decreased risk of ST positivity to *Parietaria* and of *Parietaria* monosensitization (RR = 0.47 and 0.41, respectively). This observation is of potential interest but should be confirmed in a different population. It has not been confirmed in the Spanish study group. However, in this group all of the DRB1*03 responders were also DRB1*11 or DRB1*01 and responsiveness could reflect codominant expression of the immune response gene function [30].

It is becoming increasingly clear that at least two sets of genes control allergy: HLA-linked immune response genes regulating specific responsiveness and "atopy genes" regulating IgE expression [31, 32]. Allergy is a complex disease and it is possible that the clinical definition aggregates different phenotypes and different pre-

TABLE 3 Spanish patients: prevalence of DRB1* alleles in responders and nonresponders to Par o I

DRB1*	No. of patients with allele (%)		p^a	RR	No. of patients with allele (%)		p^a	RR
	With IgG Ab to Par o I (N = 11)	Without IgG Ab to Par o I (N = 29)			With IgE Ab to Par o I (N = 8)	Without IgE Ab to Par o I (N = 32)		
01	5 (45)	1 (4)	0.004	4.7	2 (25)	4 (13)	ns	
1500	1 (9)	2 (7)	ns		1 (13)	2 (6)	ns	
1600	0	0			0	0		
03	5 (46)	9 (32)	ns		4 (50)	10 (31)	ns	
04	0	4 (14)	ns		0	4 (13)	ns	
07	6 (55)	21 (75)	ns		6 (75)	21 (75)	ns	
1100	4 (36)	1 (4)	0.02	4.0	4 (50)	1 (3)	0.002	7
1200	1 (9)	2 (7)	ns		1 (13)	2 (6)	ns	
1300	0	6 (21)	ns		0	6 (19)	ns	

^a p , Yates corrected χ^2 analysis.

disposing genetic factors. It is becoming increasingly clear that the postulated "atopy genes," controlling the atopic phenotype (i.e., the tendency to become sensitized to several different allergens accompanied by high total serum IgE level) regulate the immune response to allergens through noncognate mechanisms. It has been proposed that the effect of HLA-linked immune response genes can only be detected in allergic subjects where the effect of atopy genes is not predominant, such as patients monosensitized with low total serum IgE levels [31, 33]. This hypothesis is in agreement with our observation that the proportion of DR1101/1104 is increased in *Parietaria* monosensitized subjects. A similar observation has been reported in studies on the association of class II alleles with responsiveness to the insect allergen Chi t 1 and to the birch pollen allergen Bet v 1 [8, 10]. The increased proportion of DR 1101 and/or 04 observed in patients who had IgG but not IgE Ab towards Par o 1 (100% in a small subset of six patients who were ST negative to *Parietaria*) also suggests that mechanisms enhancing IgE synthesis may confound the effect of class-II-linked immune response genes.

There is a general agreement that in determining individual susceptibility to allergy, the genetic risk associated with HLA class II alleles plays a minor role with respect to IgE regulating genes [13]. However, there are conditions which may increase class-II-associated risk to develop specific allergies. The prevalence of the associated allele in a given population is an important factor. Thus, in the Italian study group, DR-B1*1101 and/or 04 contribute to the risk of ST positivity to *Parietaria* and the presence of IgE Ab response towards Par o 1 was irrelevant (1.2 and 1.3, respectively), whereas in the Spanish study group, deriving from a population where these alleles are known to be comparatively less frequent (11th International HLA Workshop Data Analysis. Book II Addendum), the corresponding values were RR = 3.1 and 7.0, respectively. Our data suggest that an important factor may be represented also by the prevalence of monosensitization among patients with a specific allergy.

Although presently it cannot be excluded that the primary association of responsiveness to Par o 1 is with alleles at loci which are in linkage disequilibrium with the associated DRB1 alleles, a possible interpretation of the data presented is that peptidic fragments produced by processing of Par o 1 have higher binding affinity for the products of the associated alleles (determinant selection). This hypothesis can be verified analysing in vitro HLA class II restriction requirements of Par o 1 peptide specific T-cell clones and/or determining the binding affinity of Par o 1 derived peptides for HLA-DR proteins. A further prediction is that, in family-based studies, responsiveness is in genetic linkage with the associated alleles and is inherited as a dominant character.

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