Pediatr Allergy Immunol 1996: 7: 28–34
Printed in Denmark – all rights reserved

Copyright © Munksgaard 1996
PEDIATRIC ALLERGY AND IMMUNOLOGY
ISSN 0905-6157

Mite allergy, clinical atopy, and restriction by HLA class II immune response genes

Stephan V, Schmid V, Frischer T, Sparholt S, Forster J, Wahn V, Kuehr J. Mite allergy, clinical atopy, and restriction by HLA class II immune response genes.
Pediatr Allergy Immunol 1996: 7: 28–34. © Munksgaard 1996.

From a community-based study cohort of 1812 elementary schoolchildren we selected 129 unrelated participants to investigate the relevance of HLA-class II molecules (DPB, DQB, and DRB) to the regulation of immune response to the mite allergen Der p 1 and to clinical atopic disorders. On the basis of skin prick test results validated by measurement of specific IgE, individuals were selected and divided into three groups: group I (n = 20), controls without detectable specific IgE to common inhalant allergens; group II (n = 22), children sensitized only to non-mite allergens; group III (n = 85), children sensitized to Der p 1. Clinical history of asthma, eczema, and hay fever was ascertained using standardized questionnaires. In total, 43 different HLA class II alleles (DPB, n = 19; DQB, n = 14; and DRB, n = 10) were determined by sequence-specific oligonucleotide typing with PCR-amplified DNA. We were not able to demonstrate significant differences in gene frequencies of any HLA class II allele between the group of mite-sensitized children and one of the other two groups. However, the presence of certain DRB- and DPB-haplotypes (DRB *0100/ *0300/ *1100 and DPB *0201/ *0401) was significantly associated ($p \leq 0.01$) with a history of asthma, hay fever, and atopy (defined as a history of asthma and/or hay fever and/or eczema). Other haplotypes, including DQB *0303/ *0503, DRB *0200/ *0700, and DPB 0402 were negatively associated with a history of eczema, hay fever, and atopy ($p \leq 0.01$). Thus, our findings do not suggest a relevance of HLA-class II molecules to mite allergy; however, some HLA class II haplotypes appear to be predictive of the incidence of atopic disorders.

V. Stephan[1], V. Schmid[1], T. Frischer[2], S. Sparholt[3], J. Forster[2], V. Wahn[1], J. Kuehr[2]

Departments of Pediatrics, [1]Heinrich-Heine University, Düsseldorf, Germany; [2]University Children's Hospital, Freiburg, Germany; [3]ALK Research Group, Horsholm, Denmark

Dr. Volker Stephan, Heinrich-Heine University, Düsseldorf, Department of Pediatrics, Moorenstr. 5, 40225 Düsseldorf, Germany

Key words: HLA; MHC; mite allergy; immune response genes; atopy.

Received March 7, 1995
Accepted October 28, 1995

Exposure to mite allergens is an important etiologic factor in the development of allergic rhinitis, bronchial asthma, and eczema in specifically sensitized children (1–3). Many studies have provided evidence of both environmental and hereditary aspects of the IgE immune response to inhaled allergens (4, 5). The HLA gene complex is known to contribute to the genetic susceptibility of individuals to a variety of allergic and autoimmune diseases since it controls antigen presentation by monocytes and macrophages to helper T cells (6). Previously, associations between HLA-D types and immune response to inhalant allergens such as the rye allergens (Lol p 1–3) and ragweed antigens (Amb a 5, Amb t 5) have been reported (7, 8). However, these allergens play only a minor role in the pathogenesis of childhood asthma. The Der p 1 allergen has been extensively characterized, and an immunologically standardized allergen extract is available for *in vivo* and *in vitro* assessment of immune response. In *in vitro* studies, T helper cells restricted by HLA-DP and -DR molecules were demonstrated to recognize epitopes located on group 1 allergens of the mite *Dermatophagoides pteronyssinus* (9, 10). Regarding population studies, there are preliminary reports indicating increases of HLA-DPB antigens in atopic individuals sensitized to a variety of different allergens including group 1 and 2 allergens of *D. ptero-*

Abbreviations: Der p 1: major allergen 1 of *Dermatophagoides pteronyssinus;* Der p: *D. pteronyssinus* allergen; Der f: *D. farinae* allergen; HLA: human leukocyte antigen; IgE: immunoglobulin E; PCR: polymerase chain reaction.

nyssinus (11, 12). In addition to its clinical relevance to the development of allergic disease in children, dust mite allergen is of particular interest since identification of genetic risk factors (such as the presence of predisposing immune response genes) could be helpful in initiating allergen exposure avoidance measures that have been shown to reduce clinical severity (1, 13).

In the framework of a large, community-based study, 129 9-year-old unrelated children were selected to investigate the relevance of HLA-DR, HLA-DQ, or HLA-DP molecules to an IgE-mediated immune response to Der p 1 and to a clinical history of asthma, hay fever, and eczema.

Material and methods

Study population

The data was collected in a longitudinal study which was designed to assess risk factors for allergy and asthma in children. In order to obtain a population-based sample of primary schoolchildren, all parents whose children started elementary school in 1988 were invited to participate. Questionnaires were distributed to 2604 families and were returned by 1812 families (proportion of responders 70%). Data from the first cross section are presented in detail elsewhere (14). Three consecutive skin prick tests, each 12 months apart, were carried out in spring 1990 (n = 1470), 1991 (n = 1416), and 1992 (n = 1305). Measurement of exposure to Der p 1 refers to visits to the households between December and March 1990/91, when dust samples were taken from each child's mattress. The study was approved by the local ethics committee and by local school authorities. At the end of a 2-year observation period children with a complete data set of three skin prick tests showing consistent sensitization and a random sample of those without any sensitization (total n = 198) were asked to participate in blood sampling for the current analysis. Of 138 participating children (70%), HLA class II typing and measurement of serum IgE could be carried out in 131.

Questionnaire

A first (1990), second (1991), and third questionnaire (1992) were self-administered by the children's parents. For the analyses, questions of interest were bronchial asthma (lifetime prevalence of doctor's diagnosis of asthma or of recurrent wheezy bronchitis); hay fever (lifetime prevalence of doctor's diagnosis); and eczema (lifetime prevalence of skin-reaction in the bends of the elbows and knees and on the cheeks (also called "neurodermatitis" or "eczema").

Measurement of Der p 1 exposure

Mattress dust was collected by vacuum cleaner as described previously (15). Extraction and quantification of the Der p 1 allergen were performed using species-specific antibodies to Der p 1 with an ELISA system (ALK, Denmark) according to the manufacturer's recommendations.

Skin prick testing

The procedure was carried out in the same manner on each of the three occasions according to standard procedures described previously (14). Allergen extracts (ALK, Denmark) from two mite allergens (Der p and Der f) as well as five non-mite allergens (grass, birch, and hazel pollens, and cat and dog dander) were used (concentration: 10 histamine equivalent units in prick testing). The reactions were recorded after 15 min. The largest perpendicular diameter of a wheal was measured and the arithmetic mean calculated. The diameter of the reaction to the negative control was subtracted from the allergen value. Sensitization requires a wheal to the allergen $\geq$ 2 mm as well as a ratio (wheal to allergen / wheal to histamine) $\geq$ 0.5. The validity of the skin prick test results was checked by measurement of specific serum IgE.

Specific serum IgE

Measurement of serum IgE directed against a mixture of inhalant allergens (Phadiatop, Kabi-Pharmacia, Germany), Der f, grass, birch, and hazel pollens, cat and dog dander (CAP system, Kabi-Pharmacia), and Der p 1 (Magic Lite system, ALK, Denmark) was used. IgE detection in the CAP system was evaluated in six classes with increasing serum IgE concentrations.

Group criteria based on specific sensitization

Sensitization to Der p was defined by positive skin prick test to Der p (> 90% consistent with measurement of serum IgE to Der p 1). *Sensitization to non-mite allergens was defined by* a) positive skin prick test; b) in the case of small wheal reactions to a non-mite allergen, detection of IgE antibodies against the same allergen (at least CAP class > 1); or c) in the case of a negative skin prick test, detection of IgE antibodies (CAP class $\geq$ 1) against one of the five non-mite allergens (grass, birch, and hazel pollens, cat and dog dander). *Group I (n = 22 nonatopic controls):* children with sensitization to none of the seven inhalant allergens in one of the three skin prick tests and negative specific serum IgE screening (Phadiatop) and high mite allergen exposure (Der p 1 > 10

µg/g). *Group II (n = 22):* children with sensitization to at least one of the five tested non-mite allergens. In this group sensitization to Der f was excluded by the skin prick test result and the specific serum IgE measurement. *Group III (n = 85):* children with sensitization to Der p alone (n = 10) or combined with sensitization to non-mite allergens (n = 75).

HLA typing

Genomic DNA samples were isolated from peripheral blood after boiling of cell suspensions for 5 min and recovery of cell supernatants after centrifugation as described elsewhere (16). Nucleotide sequences of primers used for amplification of the second exons of DPB1 (2 DPBAMP-A, 2 DPBAMP-B), DQB1 (2 DQBAMP-A, 2 DQBAMP-B), and DRB 1, 3, 4, and 5 (2 DRBAMP-A, 2 DRBAMP-B) have been reported by the 11th International Histocompatibility Workshop (17). PCR gene amplification was performed with 0.1 µg of genomic DNA using the GeneAmp® PCR Reagent Kit (Perkin Elmer Cetus, Überlingen, Germany). For amplification of DQB1 and DRB1 exons, samples were subjected to a total of 30 cycles consisting of 20 s at 95°C (DNA denaturing), 40 s at 55° (primer annealing), and 60 s at 72° (primer extension). A modified protocol was used for DRB1 amplification: DNA denaturing for 20 s at 95°C, primer annealing for 40 s at 65°, and extension for 60 s at 68°. To ensure complete final extension, all samples were incubated for an additional 7 min at 72°C after the last cycle (19). To assess the quantity and purity of the amplified product, 5 µl of each PCR product was run on a 1.5% agarose-minigel and stained with ethidium bromide. Following amplification, 5-µl samples were denatured in 0.4 NNaOH and then applied on Hybond N™ nylon membranes (Amersham, Braunschweig, Germany) using a 96-well dot-blot apparatus (BioRad, München, Germany).

Analysis of the second exons of the HLA-DPB1, HLA-DQB1, and HLA-DRB1, 3, 4, and 5 genes was performed using sequence-specific oligonucleotides as reported at the 11th International Histocompatibility Workshop (18). Oligonucleotides (10 pmol) were 5'-end labeled by T4-polynucleotide kinase (Boehringer Mannheim, Germany) with (γ32)-ATP (Amersham, Braunschweig, Germany) and purified on a Biospin 6 column (BioRad, München, Germany). Membranes were prehybridized at 50°C in hybridization buffer containing 5 × SSPE, 5 × Denhardt's solution, and 0.7% SDS. After 10 min, the ^{32}P-labeled probe was added to the sample, and hybridization was allowed to continue for 2 h at 50°C. Then membranes were rinsed for 10 min at room temperature with a solution of 2 × SSPE and 0.1% SDS followed by a second washing step using tetramethylammonium chloride washing solution containing 3 M tetramethylammonium chloride, 50 mM Tris-HCl (pH 8.0), 2 mM EDTA (pH 8.0), and 0.1% SDS. The final stringent wash was carried out in tetramethylammonium chloride solution at specific temperatures for each sequence-specific oligonucleotide (46–58°C). DRB1-sequence-specific oligonucleotides were 3'-end labeled with alkaline phosphatase using the Lightsmith™ II Luminescence Engineering System for oligonucleotides (Promega, Madison, WI, USA) according to the manufacturer's recommendations. The set of DRB 1-specific oligonucleotides was designed to allow for generic typing of DRB1, 3, 4, and 5 alleles. Hybridization signals were visualized by autoradiography at –70°C using Kodak XAR films and intensifying screens for 4–48 h.

Reference DNA was prepared from B-cell lines reported at the 10th International Histocompatibility Workshop and kindly provided by Prof. Grosse-Wilde, Institute of Immunology, University of Essen, Germany.

Statistical analysis

Regarding HLA class II phenotypes, the gene frequency was calculated as follows: gene frequency (%) = (sa / N) × 100. The sum of alleles (sa) was derived by counting the alleles (in homozygotes the allele was counted only once). N was the number of individuals analyzed.

For analysis of groups of alleles, the presence of one allele (and two alleles) of the respective allele group was required. To evaluate differences in allele or gene frequencies between the study groups, chi square tests were applied. The statistical procedures were performed with the Statistical Analysis System (SAS).

Results

Relationship between HLA-DR, -DQ, and -DP and sensitization to Der p

HLA-DR, -DQ, and -DP alleles were determined in 129 subjects. Typing of all six alleles was complete in 115 children, but in four participants typing of one allele was missing (DPB, n=1; DQB, n=3). Determination of both alleles could not be performed in 8 children (DQB, n=7; DRB, n=3).

In our first approach, possible associations between sensitization to Der p 1 allergen and HLA-DR, -DQ, and -DP markers were compared. Using 23 different SSO, 19 different DPB alleles could be distinguished. No statistically significant difference between the three study groups could be demonstrated for any allele (Table 1). The highest percentage of DPB1 *0401 and DPB1 *0201 were found in the group of individuals without detectable specific IgE.

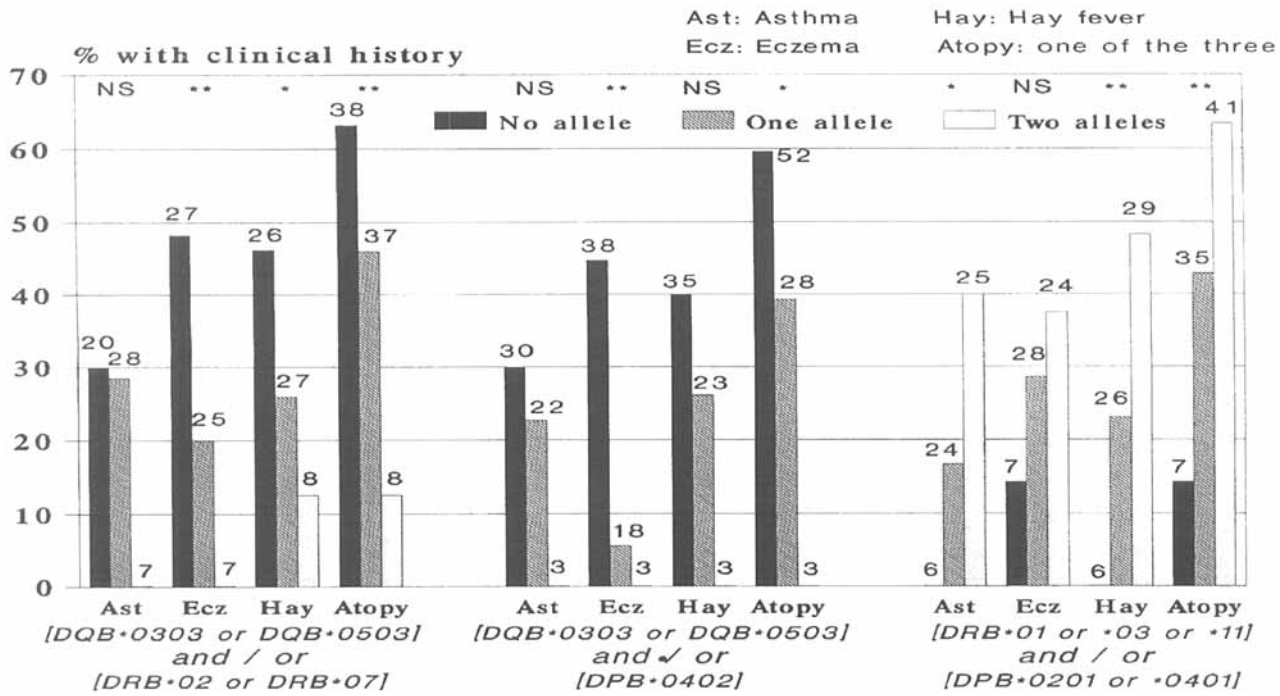

Fig. 1. The association of HLA haplotype segments and the clinical history of atopic disorders (ast: asthma; ecz: eczema; hay: hay fever; atopy: at least one of the three disorders) in SPT-positive children. Numbers indicate the total number of children represented by the respective columns. NS, not significant; * $p < 0.05$; ** $p < 0.01$.

In addition we examined whether there was an association between the presence of HLA-DPB alleles and a clinical history of atopic disease. DPB *0201 and *0401 were both found to occur more frequently in sensitized children with a clinical history of atopic disease whereas DPB *0402 was found more frequently in children sensitized to Der p without clinical signs of atopic disease. Differences between both groups did not show statistical significance.

Fourteen different DQB alleles were distinguished using 16 sequence-specific oligonucleotides. No significant difference was found among HLA-DQB gene frequencies of any of the three study groups (Table 2). DQB *0303 and *0503 were both found to occur more frequently in sensitized children without a clinical history of atopic disease; however, differences were not statistically significant.

Ten DRB1 specificities were determined (Table 3). Gene frequencies of the supertypic specificities DRw52 and DRw53 are not listed as they were found to be associated with their respective DRB1 counterparts as expected. Gene frequencies of HLA-DR alleles among the three study groups did not differ significantly. In children sensitized to Der p (group III) and presenting with a clinical history of atopic disease (asthma, hay fever, eczema), DRB *0100, *0300, and *1100 were found to be increased and DRB *0200 and *0700 decreased compared to those Der p-sensitized participants without such symptoms.

Relationship of HLA-haplotype segments to clinical history of atopic disease

Positive and negative associations between certain HLA alleles and a clinical history of atopic disor-

Table 1. The gene frequencies (%) of 10 group-specific HLA-DRB alleles in 127 schoolchildren. No statistically significant differences between the study groups; specificities DRw52 and DRw53 are not listed as they were found to be associated with their respective DRB1 counterparts, as reported previously

Group/population	DPB1 – ALLELE																		
	0101	0201	0202	0301	0401	0402	0501	0601	0901	1001	1101	1301	1401	1501	1701	1901	2301	2901	3601
Total population (n = 129)	5.4	27.1	1.6	17.1	65.9	27.9	3.9	6.2	0.8	4.7	3.1	2.3	4.7	0.8	3.1	1.6	0.8	0.8	1.6
Controls (n = 22)	4.6	40.9	0	9.1	81.8	22.7	0	4.6	0	0	0	0	9.1	0	4.6	9.1	0	0	0
Sensitization to non-mite allergens (n = 22)	4.6	18.2	0	2.8	63.6	18.2	9.1	9.1	0	4.6	4.6	4.6	4.6	4.6	0	0	0	0	4.6
Sensitization to mite allergens (n = 85)	5.9	25.9	2.4	17.7	62.4	31.8	3.5	5.9	1.2	5.9	3.5	2.4	3.5	0	3.5	0	1.2	1.2	1.2
All sensitized children (n = 107)	5.6	24.3	1.9	18.7	62.6	29.0	4.7	6.5	0.9	5.6	3.7	2.8	3.7	0.9	2.8	0	0.9	0.9	1.9
Sensitization and no clinical sign (n = 41)	5	19.5	0	19.5	58.5	34.2	4.9	4.9	2.4	7.3	7.3	7.3	7.3	0	4.9	0	2.5	0	2.4
Sensitization and clinical sign (n = 42)	0	28.6	4.8	19.1	71.4	23.9	7.1	4.8	0	7.1	0	0	0	2.4	0	0	0	0	2.4

Table 2. Gene frequencies (%) of 14 different HLA-DQB alleles in 124 schoolchildren. No statistically significant differences between the study groups could be demonstrated for any allele

Group/population	DQB1 – ALLELE													
	0201	0301	0302	0303	0401	0501	0502	0503	0593	0601	0602	0603	0604	0694
Total population (n = 124)	41.9	32.3	20.2	11.3	7.3	20.2	4.0	4.0	3.2	3.2	25.0	12.1	4.8	0.8
Controls (n = 20)	55	15	15	15	5	30	5	0	5	0	35	10	10	0
Sensitization to non-mite allergens (n = 21)	33.3	23.8	23.8	14.3	14.3	4.8	9.5	14.3	4.8	9.5	23.8	4.8	4.8	0
Sensitization to mite allergens (n = 83)	41	38.6	20.5	9.6	6.0	21.7	2.4	2.4	2.4	2.4	22.9	14.5	3.6	1.2
All sensitized children (n = 104)	39.4	35.6	21.2	10.6	7.7	18.3	3.9	4.8	2.9	3.9	21.1	12.5	3.9	1
Sensitization and no clinical signs (n = 40)	40	30	20	12.5	10	15	7.5	12.5	2.5	2.5	27.5	10	2.5	0
Sensitization and clinical signs (n = 39)	46.2	35.9	18	5.1	7.7	23.1	0	0	2.6	2.6	23.1	18	5.1	2.6

ders were analyzed to identify combinations of HLA-DR, -DQ, and -DP alleles indicating increased or decreased risk of clinical disease. The frequencies of clinical manifestation stratified for combinations of HLA-DR, HLA-DQ, and HLA-DP alleles are shown in Fig. 1. Asthma, hay fever, and atopy (defined as occurrence of asthma and/or hay fever and/or eczema) were more frequent in the sensitized individuals with HLA molecules DRB *0100/*0300/*1100/DPB *0201/*0401 than in sensitized children without these alleles. In 15 out of 35 (43%) and 26 out of 41 children (64%) with, respectively, one and two of these alleles, a history of at least one of the clinical signs was reported, whereas this was the case in only 1 of 7 children (14%) without any of the mentioned HLA alleles. The association was statistically significant for asthma ($p \leq 0.05$), hay fever ($p \leq 0.01$), and atopy ($p \leq 0.01$). Lower prevalences of eczema ($p \leq 0.01$), hay fever ($p \leq 0.05$), and atopy ($p \leq 0.01$) were seen in cases with alleles DQB *0303/*0503/DRB *0200/*0700. In addition, a negative association was found between DRB *0200/*0700/DPB *0402 and a clinical history of eczema ($p \leq 0.01$) and atopy ($p \leq 0.05$).

Discussion

In a sample of sensitized and nonsensitized individuals derived from a community-based cohort of 10-year-old children, associations between the HLA class II genotype and allergic sensitization, as well as clinical history of atopy, were analyzed. Particular attention was paid to dust mite allergy as the universally most common specific sensitization which is closely related to perennial inhalant allergy (2). When selecting the participants, a minimum Der p 1 concentration of 10 µg/g in mattress dust was required for controls in order to exclude the possibility that low allergen exposure had prevented sensitization in some cases. On the basis of skin prick test validated by specific IgE measurement, sensitization was excluded in the controls and ensured in the group with sensitization to non-mite allergens, as well as in the group of children sensitized to Der p.

We were not able to demonstrate a statistically significant difference of the gene frequencies between controls and Der p-sensitive children; thus, IgE-response to Der p 1 appears not to be restricted

Table 3. Gene frequencies (%) of 19 different HLA-DPB alleles in 129 schoolchildren. No statistically significant differences between the study groups could be demonstrated for any allele

Group/population	DRB1 – ALLELE									
	01	02	03	04	06	07	08	10	11	12
Total population (n = 127)	16.5	31.5	22	29.1	23.6	25.2	10.2	3.1	22.8	4.7
Controls (n = 21)	14.3	38.1	28.6	23.8	19.0	28.6	14.3	9.5	14.3	0
Sensitization to non-mite allergens (n = 22)	9.1	36.4	22.7	22.7	27.3	18.2	13.6	0	18.2	9.1
Sensitization to mite allergens (n = 84)	19.1	28.6	20.2	32.1	23.8	26.2	8.3	2.4	26.2	4.8
All sensitized children (n = 106)	17	30.2	20.8	30.2	24.5	24.5	9.4	1.9	24.5	5.7
Sensitization and no clinical signs (n = 40)	15	32.5	17.5	25	25	32.5	10	0	22.5	5
Sensitization and clinical signs (n = 42)	23.8	23.8	26.2	28.6	26.2	19.1	9.5	2.4	31	2.4

by any of the different HLA alleles tested. Since HLA-typing data in a normal population with the appropriate ethnic background was available only for HLA-DPB (19), a nonatopic and a non-mite-sensitized control group were included in this study. The lack of an association between HLA alleles and immune responsiveness could be due to the fact that most Der p-sensitive children were additionally skin prick test-positive to other antigens. However, analysis of the HLA pattern of children in group III with exclusive sensitization to mite allergens (n = 10) was not different from the entire group of Der p-sensitive children. Recently, studies on the molecular genetics of immune responsiveness to the mite allergen Der p 1 have yielded promising results. Higgins et al. demonstrated that T cells restricted by HLA-DP molecules are able to recognize epitopes located on group I allergens of Der p (9). In addition, there are two communications demonstrating increases of DPB1 *0401 frequencies in atopic individuals sensitized to a variety of different allergens including group I and II allergens of Der p (11, 12). However, the highest percentage of DPB1 *0401 and DPB *0201 alleles were in our control group. We cannot exclude the possibility that the high frequency of these alleles in the control group is coincidentally due to the small number of controls; however, allele frequencies found in our study population matched exactly results obtained previously by other investigators in unselected individuals from the same geographical region (19). The lack of association of a single HLA-allele with IgE response to Der p 1 may be due to the fact that a number of different epitopes can be distinguished on the Der p 1 molecule. Therefore, different HLA alleles responsible for recognition of different allergen epitopes could be involved in presentation of the Der p 1 allergen to T cells.

In the second part of our study we provide evidence for the association of certain HLA class II haplotypes with the absence or presence of a clinical history of atopic disease. Previous studies on the association of HLA gene products and atopic diseases have yielded conflicting results. Population studies in patients with bronchial asthma demonstrated increased HLA-B5 and decreased B17 (20), increased HLA-A1 and B8 (21, 22), decreased HLA-B12 (21), and increased HLA-A2 and decreased HLA-B8 (23). In addition, there are reports of increases of HLA-A1 and B5 in patients with eczema complicated by asthma and/or hay fever (24) and increases in HLA-A3 and B7 in patients with hay fever subsequently developing bronchial asthma (25). Regarding HLA class II gene products, two earlier studies failed to find associations of HLA antigens with bronchial asthma (21, 26). In two recent reports relying on PCR technology, HLA-DQw2 was found to be relatively increased (27) and DPB *0401 significantly decreased in Chinese and South American mulatto asthmatic subpopulations, respectively (28). However, these results must be compared with a Caucasian population with caution since the different ethnic background results in great variations of the distribution of HLA antigens in the normal population. In our study no single HLA allele was found to be significantly increased in the atopic population. However, in screening combinations of the most prevalent alleles, we identified a number of HLA haplotype segments that were either predisposing to or protective against atopic disorders. The association of the different HLA alleles composing the haplotype segments cannot be explained by linkage disequilibrium and is therefore due to random assortment. In a similar approach Blumenthal et al. have demonstrated the importance of HLA haplotypes for the development of rhinitis or asthma in ragweed-sensitive subjects (29). In our study, the presence of HLA alleles did not distinguish between different disease manifestations but was merely indicative of the presence or absence of clinical disease. However, it must be pointed out that, due to the relatively small number of patients in our study, additional associations may have been missed. At present, it is not clear how the presence of HLA alleles affects susceptibility to atopic disease. Both stimulating and suppressive effects of HLA-DR genes on IgE-mediated disease have been described previously (29, 30). The results of the present study suggest that genes of the DP and DQ locus can act in a similar way.

In conclusion, our results are evidence of the importance of HLA alleles in the development of atopic disorders. However, this and other studies clearly indicate that other factors, both environmental and non-HLA genetic, may be involved in the pathogenesis of Der p 1 sensitization and subsequent clinical disease.

Acknowledgements

We wish to thank Dr. D. G. Marsh for his advice and help in establishing DNA-typing procedures. We also extend our thanks to the members of the HLA and Allergy Workshop supported by the European Science Foundation. This work was supported by the Federal Ministry for Research and Technology, Bonn, Germany, grant no. 0701565.

References

1. LAU S, FALKENHORST G, WEBER A, WAHN U. High mite allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol 1989: 84: 718–25.
2. PLATTS-MILLS TAE, DE WECK AL. Dust mite allergens and asthma – a worldwide problem. J Allergy Clin Immunol 1989: 83: 416–27.

3. SPORIK R, HOLGATE ST, PLATTS-MILLS TAE, COGSWELL JJ. Exposure to house dust mite allergen (*Der p* I) and the development of asthma in childhood. N Engl J Med 1990: 323: 502–7.
4. BLUMENTHAL M, BACH F. Immunogenetics of atopic disease. In: MIDDLETON E, REED CE, ELLIS EF, eds: Allergy: principles and practice. St. Louis, MO: CV Mosby, 1983: 11–43.
5. MARSH DG, MEYERS DA, BIAS WB. The epidemiology and genetics of atopic allergy. N Engl J Med 1981: 305: 1551–9.
6. KOSTYU DD, AMOS DB. The HLA complex: genetic polymorphism and disease susceptibility. In: SCIVER CR, BEAUDET AL, SLY WS, VALLE D, eds. The metabolic basis of inherited disease. New York: McGraw-Hill, 1989: 225–49.
7. ANSARI AA, SHINOMIYA N, ZWOLLO P, MARSH DG. HLA-D gene studies in relation to immune responsiveness to a grass allergen *Lol p* III. Immunogenetics 1991: 33: 24–32.
8. ROEBBER M. Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergens *Amb a* VI (Ra 6) is associated with HLA-DR 5 in allergic humans. Immunogenetics 1987: 26: 230–6.
9. HIGGINS JA, LAMB JR, MARSH SGE, et al. Peptide induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite). J Allergy Clin Immunol 1992: 90: 749–56.
10. O'HEHIR RE, LAMB JR. Direct evidence for a functional role of HLA-DRB1 and -DRB3 gene products in the recognition of *Dermatophagoides* spp. (house dust mite) by helper T lymphocytes. Int Immunol 1990: 2: 885–92.
11. EURA M, FREIDHOFF LR, KAUTZKY EE, et al. HLA-DP and IgE specific response allergens. J Allergy Clin Immunol 1992: 89: 218.
12. YOUNG RP, SCHOU C, MATTHIESEN F, et al. HLA-DP restriction to purified allergens. Allergy 1992: 47 (Suppl): 70.
13. PLATTS-MILLS TAE, TOVEY ER, MITCHELL EB, MOSZORO H, NOCK P, WILKINS SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982: 2: 675–8.
14. KUEHR J, FRISCHER T, KARMAUS W, et al. Early childhood risk factors for sensitization at school age. J Allergy Clin Immunol 1992: 90: 358–63.
15. KUEHR J, FRISCHER T, KARMAUS W, et al. Natural variation in mite antigen density in house dust and relationship to residential factors. Clin Exp Allergy 1994: 24: 229–37.
16. HOWELL WM, SAGE DA, HAEGERT DG, EVANS PR, SMITH JL. PCR-SSO typing for HLA-DPB alleles. Eur J Immunogen 1991: 18: 81–95.
17. 11th International Histocompatibility Workshop protocol for the HLA-DNA typing technique, Yokohama, Japan (1992).
18. IVINSON AJ. Optimizing PCR conditions for HLA class II SSO typing. Eur J Immunogen 1991: 18: 23–32.
19. EIERMANN TH, FAKLER J, MULLER CR, BALLAS M, GOLDMANN SF. HLA-DPB1 oligonucleotide typing of a southwest German Caucasian population. Tissue Antigens 1991: 38: 193–8.
20. HUANG LS, HSIEH KH, LIOU SY. HLA and childhood asthma. Chin J Microbiol Immunol 1981: 14: 102–6.
21. MORRIS MJ, FAUX J, TING A, MORRIS BJ. HLA-A, B and C and HLA-DR antigens in intrinsic and extrinsic asthma. Clin Allergy 1980: 10: 173–8.
22. THORSBY E, ENGESET A, LIE SO. HLA antigens and susceptibility to diseases. A study of patients with acute lymphoblastic leukemia, Hodgkin's disease and childhood asthma. Tissue Antigens 1971: 1: 147–51.
23. RACHELEFSKI G, TERASAKI PI, PARK MS, KATZ R, SIEGAL SAITO S. Strong association between B-lymphocyte group-2 specificity and asthma. Lancet 1976: 2: 1042–4.
24. TURNER M, BROSTOFF J, WELLS R, STOKES C, SOOTHILL J. HLA in eczema and hay fever. Clin Exp Immunol 1977: 27: 43–9.
25. GEERTS SJ, POTTGENS H, LIMBURG M, VAN ROOD J. Predisposition for atopy or allergy linked to HLA. Lancet 1975: 1: 461–3.
26. BRUCE CA, BIAS WA, NORMAN PS, LICHTENSTEIN LM, MARSH DG. Studies of HLA antigen frequencies, IgE levels, and specific allergic sensitivities in patients having ragweed hay fever, with or without asthma. Clin Exp Immunol 1976: 25: 67–73.
27. HSIEH KH, SHIEH CC, HSIEH RP, LIU WJ. Association of HLA-DQw2 with Chinese childhood asthma. Tissue Antigens 1991: 38: 181–2.
28. CARABALLO L, MARRUGO J, JIMINEZ S, ANGELINI G, FERRARA GB. Frequency of DPB1 *0401 is significantly decreased in patients with allergic asthma in a mulatto population. Human Immunol 1991: 32: 157–61.
29. BLUMENTHAL M, MARCUS-BAGLEY D, AWDWH Z, JOHNSON B, YUNIS EJ, ALPER CA. (HLA-B7, SC31, DR2) and (HLA-B8, SC01, DR3) haplotypes distinguish subjects with asthma from those with rhinitis only in ragweed pollen allergy. J Immunol 1992: 148: 411–16.
30. MARSH DG. Immunogenetic and immunochemical factors determining immune responsiveness to allergens: studies in unrelated subjects. In: MARSH DG, BLUMENTHAL MN, eds. Genetic and environmental factors in clinical allergy. University of Minnesota Press, 1990: 97–124.