

Association Between HLA-DRB3*0101 and Immunoglobulin-E Responsiveness to *Bet v* I

Susanne H. Sparholt, Jørgen Georgsen, Hans O. Madsen, Ulrik G. Svendsen, and Carsten Schou

ABSTRACT: The relationship between HLA-DRB3 antigens and specific IgE responsiveness to highly purified major allergen from white birch (*Betula verrucosa*), *Bet v* I, was studied in 43 patients, who were skin-prick test positive to birch pollen extract. Specific IgE to *Bet v* I was measured using an assay based on the principle of the Magic Lite method. HLA-DR and -DQ typings were performed by standard restriction fragment-length polymorphism techniques and HLA-DRB3 typing by the use of polymerase chain reaction and sequence-specific oligonucleotide. Of the 43 patients, 41 (95%) exhibited *Bet v* I-specific IgE. The data showed a significant association

between HLA-DRB3*0101 and specific IgE to *Bet v* I ($p_c = 0.02$). Furthermore, it was shown that this association was linked to the overall IgE responsiveness and not to a limited area of IgE sensitivity. The present study confirms and further identifies the association of DRB3*0101/0301 earlier described in an Austrian population to be present also in a Danish study group. This underlines the importance of this particular allele in the allergic immune response to birch pollen and emphasizes the importance of investigating the molecular basis of human allergic immune responsiveness. *Human Immunology* 39: 76–78 (1994)

ABBREVIATIONS

Ig immunoglobulin

PMP paramagnetic particle

INTRODUCTION

The relationship between HLA class II antigens and human IgE responsiveness to highly purified antigens has been the topic of several studies [1–4]. Most of these investigations have dealt with the association of HLA-DRB1 antigens to specific IgE responsiveness. However, O'Hehir et al. [5, 6] have demonstrated the functional role of HLA-DRB3 gene products in the restriction of a panel of human T-cell clones specific to major allergens from *Dermatophagoides* spp. Recently, a study of an Austrian birch allergic population documented a significantly higher frequency of the HLA-DRB3 alleles, DRw52a/c (DRB3*0101 and *0301), in *Bet v* I IgE responsiveness [7]. In this paper the relation-

ship between HLA-DRB3 antigens and specific IgE to *Bet v* I has been investigated. The aim was to analyze whether this association could be confirmed in a Danish birch allergic population and whether a possible association could be ascribed to either one of the two alleles.

MATERIALS AND METHODS

The study group consisted of 43 Caucasians (27 women and 16 men) from Copenhagen and suburbs with clinically manifested allergy to birch pollen. They were selected according to positive skin prick test to birch pollen extract (Soluprick SQ; ALK, Denmark).

The distribution of the individual HLA markers was compared with that of a control group that consisted of 182 unrelated healthy adults from Copenhagen.

HLA-DR and -DQ typings were performed by conventional restriction fragment-length polymorphism techniques [8]. The HLA-DRB3 alleles were identified according the method of Bidwell [9] and Bidwell and Bignon [10], who described the linkage between specific

From ALK Research (S.H.S., C.S.), Hørsholm; the Department of Clinical Immunology (J.G.), Odense University Hospital, Odense; the Department of Clinical Immunology (J.G., H.O.M.), Rigshospitalet, University of Copenhagen, Copenhagen; and the Department of Medicine Y (U.G.S.), Gentofte County Hospital, University of Copenhagen, Hellerup, Denmark.

Address reprint requests to Dr. S. H. Sparholt, ALK Research, Bøge Alle 10–12, DK-2970 Hørsholm, Denmark.

Received (E) June 10, 1993; accepted August 24, 1993.

TABLE 1 Association between specific IgE responsiveness to *Bet v* I and HLA-DRB3*0101 in a Danish birch-allergic group compared with a control group from the same area

HLA marker	<i>Bet v</i> I-positive group allele frequency (%)	Control group allele frequency (%)	Relative risk value ($\pm$ 95% CL)	Fisher exact test p_c (one tail)
HLA-DRB3*0101	51.2 ($n = 41$)	29.5 ($n = 182$)	2.5 (1.3/5.0)	0.02

HLA-DRB1 alleles and HLA-DRw52a, b, and c, respectively. HLA-DRB3 typing was subsequently performed by the use of polymerase chain reaction–sequence-specific oligonucleotide hybridization method as proposed by the 11th International Histocompatibility Workshop [11].

Bet v I was prepared from aqueous extracts as described by Ipsen and Hansen [12].

Specific IgE to purified *Bet v* I was measured using an assay based on the principle of the Magic Lite SQ-specific IgE assay (ALK). Briefly, *Bet v* I was covalently coupled to paramagnetic particles (PMPs) and used as solid phase in the immunoassay. Serum sample (25 μ l) was incubated with 250 μ l PMP–*Bet v* I for 30 minutes. *Bet v* I-specific IgE bound to the solid phase was magnetically separated from the supernatant. Bound IgE was detected in a luminometer (Magic Lite Analyser; Ciba Corning Diagnostics, USA) with a monoclonal anti-human IgE (ALK) coupled to the chemiluminescence reagent, acridinium ester.

Disease association between HLA-DRB3 alleles and *Bet v* I-specific IgE responsiveness was calculated using relative risk estimates and Fisher's exact test. To correct for incidental significance, the p value was multiplied by the number of antigens compared, p_c [13].

RESULTS

Serum samples from the patients were tested for specific IgE to *Bet v* I. Of 43 patients, 41 (95%) of the skin prick test-positive individuals exhibited detectable IgE toward *Bet v* I, confirming the allergen to be a major allergen from birch pollen [14]. Only these 41 persons were considered in the HLA association analysis.

It was tested whether the frequencies of the three different HLA-DRB3 alleles (*0101, *0201/2, and *0301) in the *Bet v* I-positive group were found to be significantly higher than those in the control group. Both the chi-squared test and Fisher's exact test showed comparable results. The frequency of the DRB3*0101 allele was 51.2% in the study group compared with 29.5% in the control group, and the statistical analysis showed a highly significant association between DRB3*0101 and specific IgE responsiveness to *Bet v* I; p_c was 0.02, one tailed (Table 1).

There was no significant difference in the frequencies of the HLA-DRB3*0201/2 and *0301 alleles in the two groups.

To analyze whether this significant association could be ascribed to a particular level of IgE sensitivity, the number of serum samples were divided according to *Bet v* I-specific IgE content. The frequency of the HLA-DRB3*0101 allele is listed for each group in Table 2, which shows no difference in the frequency of the DRB3*0101 allele in any of the individual IgE groups compared with the overall frequency of the allele. This indicates DRB3*0101 to be associated with overall IgE responsiveness to *Bet v* I are not linked to a limited area of IgE sensitivity.

DISCUSSION

Several studies have documented an association of different HLA class II markers and specific IgE responsiveness to purified allergen [1–4]. The present study has dealt with the role of HLA-DRB3 gene products in the allergic immune response to the major allergen from birch pollen, *Bet v* I. Fischer et al. [7] have described a significantly higher frequency of the HLA-DRB3*0101/0301 alleles in an Austrian birch-allergic study group, characterized by an IgE response directed mainly toward *Bet v* I. In this study, we compared the frequencies of the three alleles in a control group with a birch-allergic group not preselected for a particular type of IgE response. As shown in Table 1, the study confirms this

TABLE 2 Number of patient sera divided according to *Bet v* I-specific IgE content

IgE to <i>Bet v</i> I (SU/ml)	Serum samples (n)	DRB3*0101 + (n)
< 4	1	1
< 20	16	8
< 100	13	6
< 300	10	6
> 300	1	0
Total	41	21

In the third row the number of HLA-DRB3*0101-positive patients are listed for each of the IgE groups.

very strong association described by Fischer et al. [7], and furthermore documents that *Bet v* I responsiveness is linked only to the HLA-DRB3*0101 allele and not to the DRB3*0301 allele. The role of this allele in susceptibility to birch pollen allergy is further underlined by the fact that the same association is found in two independent birch allergic groups, the Austrian and the Danish.

It could be speculated that HLA association to specific IgE responsiveness would be strongly significant in patients with low amounts of IgE, as they may have been newly sensitized, and less predominant in patients with high IgE values. In Table 2, however, it was shown that the frequency of the DRB3*0101 allele in the different groups of *Bet v* I-specific IgE sensitivities were the same, indicating the allele to be associated with overall IgE responsiveness to *Bet v* I.

Fischer et al. [7] described an association between a three-amino-acid sequence from the second hypervariable region of DRB, position 30–32 (tyrosine–phenylalanine–histidine) and selective IgE responsiveness to *Bet v* I. This sequence is found in more than 10 different DRB alleles including DRB3*0101 and *0301. However, as the present study showed that *Bet v* I-specific IgE responsiveness was linked only to the HLA-DRB3*0101 allele, it was of primary relevance to look for amino acid sequences that were unique to this particular allele. The deduced amino acid sequence of the HLA-DRB3*0101 allele shows an arginine in residue 11 in the first hypervariable region. The arginine is basic and unique when compared with residue 11 of all other DRB alleles, which have either neutral or acidic amino acids at this particular residue. According to the newly published three-dimensional structure of the class II antigen, HLA-DR1, residue 11 is situated as a part of the β -pleated sheet that forms the bottom of the antigen-binding groove [15]. It would therefore be of primary interest to study the restriction of this particular allele in T-cell responsiveness to *Bet v* I and to investigate the importance of this particular arginine in peptide binding.

ACKNOWLEDGMENTS

This study was supported by grants from the Lundbeck Foundation, Denmark, and by the Danish Foundation for Medical Research, Region of Copenhagen, the Faroe Islands and Greenland.

REFERENCES

1. Ansari AA, Freidhoff LR, Meyers DA, Bias WB, Marsh DG: Human immune responsiveness to *Lolium perenne* pollen allergen *Lol p* III (Rye III) is associated with HLA-DR3 and DR5. *Hum Immunol* 25:59, 1989.
2. Friedhoff LR, Ehrlich-Kautzky E, Meyers DA, Ansari AA, Bias WB, Marsh DG: Association of HLA-DR3 with human immune response to *Lol p* I and *Lol p* II allergens in allergic subjects. *Tissue Antigens* 31:211, 1988.
3. Marsh DG, Hsu, SH, Roebber M, Ehrlich-Kautzky E, Freidhoff R, Meyers DA, Pollard MK, Bias WB: HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. *J Exp Med* 155: 1439, 1982.
4. Marsh DG, Freidhoff LR, Ehrlich-Kautzky E, Bias WB, Roebber M: Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb a* VI (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics* 26:230, 1987.
5. O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR: MHC class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 64:627, 1988.
6. O'Hehir RE, Mach B, Berte C, Greenlaw R, Tiercy J-M, Bal V, Lechler RI, Trowsdale J, 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* 2:885, 1990.
7. Fischer GR, Pickl WF, Faé I, Ebner C, Ferreira F, Breiteneder H, Vikoukal E, Scheiner O, Kraft D: Association between IgE response against *Bet v* I, the major allergen of birch pollen, and HLA-DRB alleles. *Hum Immunol* 33:259, 1992.
8. Bidwell JF, Bidwell EA, Savage DA, Middleton D, Klouda PT, Bradley BA: A DNA-RFLP typing system that positively identifies serologically well-defined and ill-defined HLA-DR and DQ alleles, including DRw10. *Transplantation* 45:640, 1988.
9. Bidwell J: DNA-RFLP analysis and genotyping of HLA-DR and DQ antigens. *Immunol Today* 9:18, 1988.
10. Bidwell J, Bignon JD: DNA RFLP typing for HLA class II. European Histocompatibility Conference, Strasbourg, March 21–23, 1990.
11. Kimura A, Sasazuki T: Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA typing technique. In Tsuji K, Aizawa M, Sasazuki T (eds): *HLA 1991*, vol 1. Oxford, Oxford University Press, 1992.
12. Ipsen H, Hansen OC: The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) *Aln g* I, birch (*Betula verrucosa*) *Bet v* I, hornbeam (*Carpinus betulus*) *Car b* I and oak, (*Quercus alba*) *Que a* I pollens. *Mol Immunol* 28:1279, 1991.
13. Tiwari JL, Terasaki PI: *HLA and Disease Associations*. New York, Springer-Verlag, 1985, p 18.
14. Ipsen H, Løwenstein H: Isolation and immunochemical characterization of the major allergen of birch pollen (*Betula verrucosa*). *J Allergy Clin Immunol* 72:150, 1983.
15. Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC: Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33, 1993.