



Association of HLA class II alleles with sensitization to cow dander Bos d 2, an important occupational allergen

Anu Kauppinen^{a,*}, Juha Peräsaari^b, Antti Taivainen^c, Tuure Kinnunen^a, Soili Saarelainen^a, Marja Rytönen-Nissinen^a, Hayley Jeal^d, Meinir Jones^d, Tuomas Virtanen^a

^a Department of Clinical Microbiology, Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland

^b Clinical Laboratory, Finnish Red Cross Blood Service, Helsinki, Finland

^c Department of Pulmonary Diseases, Kuopio University Hospital, Kuopio, Finland

^d Department of Occupational and Environmental Medicine, Faculty of Medicine, Imperial College (NHLL), London, United Kingdom

ARTICLE INFO

Article history:

Received 4 February 2011

Accepted 31 August 2011

Keywords:

Association

HLA class II

Allele

Lipocalin

Occupational allergy

ABSTRACT

Allergic sensitization results from a complex interaction between genetic and environmental factors. Earlier studies have shown that highly polymorphic HLA genes are associated with a variety of allergies. Several important respiratory allergens belong to the family of lipocalin proteins. These include occupational sensitizers, such as cow Bos d 2 or rat Rat n 1, and prevalent indoor sensitizers, such as dog Can f 1 or cockroach Bla g 4. HLA associations with sensitization to lipocalin allergens are incompletely known. In the present study we have investigated an association between HLA alleles and sensitization to the major cow allergen Bos d 2. The HLA-DR/DQ genotypes of 40 Bos d 2-sensitized subjects having occupational asthma were determined by polymerase chain reaction (PCR) and the results were compared with the genotypes of 151 unrelated Finnish subjects. The frequencies of HLA class II alleles DRB1*0101, DRB1*0404, DQB1*0302, and DQB1*0501 were significantly higher among Bos d 2-sensitized than among control subjects. In addition, the allergic subjects expressed significantly lower frequencies of HLA-DRB1*0301 and DQB1*0201 alleles than did the control subjects. These data suggest that the HLA class II alleles DRB1*0101, DRB1*0404, DQB1*0302, and DQB1*0501, and the haplotypes that include them, are associated with sensitization to the major cow allergen Bos d 2, whereas HLA-DRB1*0301 and DQB1*0201 are dissociated with it. Amino acid analysis provides a biologically plausible explanation for the HLA associations.

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Introduction

Asthma is the most common occupational respiratory disorder in developed countries (Kogevinas et al. 1999). In a study including 12 industrialized countries, farmers were found to be among the occupations with the highest risk of asthma (Kogevinas et al. 1999). In Finland, cow dander is an important work-related sensitizer and responsible for the most cases of occupational asthma among farmers (Koskela et al. 2003; Virtanen et al. 1996).

The major allergen found in bovine dander is the lipocalin protein Bos d 2 (Rautiainen et al. 1997). In addition to Bos d 2, the majority of important mammalian respiratory allergens are

lipocalins (Virtanen and Kinnunen 2008). These include, for example, major allergens of dog, cat, horse, rabbit, mouse, rat, and guinea-pig (Virtanen and Kinnunen 2008). Lipocalin proteins share a similar three-dimensional structure although their sequential identity at the amino acid level is often relatively low, between 20 and 30% (Akerstrom et al. 2000; Flower et al. 2000). One of their functions in the body is to carry hydrophobic ligands, such as pheromones and vitamins (Akerstrom et al. 2000).

Allergic sensitization results from a complex interaction between genetic and environmental factors. It has become obvious that there is no single susceptibility gene that increases the risk for allergies. Among the most investigated genes are those of human leucocyte antigens (HLA) which are the most polymorphic genes in the human genome (The MHC sequencing consortium 1999). The function of HLA class II molecules is to present peptides derived from exogenous proteins on the surface of antigen-presenting cells (APCs) to specific CD4+ T lymphocytes.

Sensitization, for example, to Art v 1 of mugwort (Jahn-Schmid et al. 2002; Torio et al. 2003) or phospholipase A₂ (PLA₂) of bee venom (Sanchez-Velasco et al. 2005) have been associated with

Abbreviations: CI, confidence interval; HLA, human leucocyte antigen; OD, odds ratio; PCR, polymerase chain reaction; SPT, skin prick test.

* Corresponding author at: University of Eastern Finland, Institute of Clinical Medicine, P.O.B. 1627, FIN-70211 Kuopio, Finland. Tel.: +358 40 3553216; fax: +358 17 162048.

E-mail address: anu.kauppinen@uef.fi (A. Kauppinen).

Table 1
Prevalence of HLA class II alleles in Bos d 2-sensitized and control subjects.

Allele	Bos d 2-sensitized subjects		Control subjects		P value	OR	95% CI
	Number	%	Number	%			
DRB1*							
0101	19	23.8	40	13.2	0.025	2.04	1.105–3.767
0102	1	1.3.0	1	0.3			
0103	0	0.0	1	0.3			
0301	2	2.50	33	10.9	0.017	0.21	0.049–0.891
0401	11	13.8	25	8.3			
0402	0	0.0	2	0.7			
0403	3	3.8	2	0.7			
0404	12	15.0	16	5.3	0.006	3.15	1.426–6.978
0408	1	1.30	5	1.7			
0701	1	1.30	20	6.6			
0801	7	8.8	26	8.6			
0802	0	0.0	1	0.3			
0901	1	1.3	12	4.0			
1001	1	1.3	4	1.3			
1101/04	2	2.50	16	5.3			
1102	0	0.0	1	0.3			
1103	0	0.0	3	1.0			
1201	3	3.8	12	4.0			
1202	0	0.0	1	0.3			
1301	7	8.8	26	8.6			
1302	1	1.30	8	2.6			
1303	0	0.0	3	1.0			
1305	0	0.0	1	0.3			
1401	0	0.0	7	2.3			
1501	8	10.0	34	11.3			
1601	0	0.0	2	0.7			
Total	80	100.0	302	100.0			
DQB1*							
0201	2	2.5	33	10.9	0.017	0.21	0.049–0.891
0202	1	1.3	15	5.0			
0301	9	11.3	44	14.6			
0302	24	30.0	43	14.2	0.002	2.58	1.450–4.597
0303	3	3.8	20	6.6			
0402	7	8.8	27	8.9			
0501	21	26.3	46	15.2	0.031	1.98	1.099–3.569
0502	0	0.0	2	0.7			
0503	0	0.0	8	2.6			
0602	8	10.0	33	10.9			
0603	5	6.3	22	7.3			
0604	0	0.0	7	2.3			
0606	0	0.0	1	0.3			
0609	0	0.0	1	0.3			
Total	80	100.0	302	100.0			

The percentages involved with statistical significance have been bold.

HLA-DRB1*01 and HLA-DRB1*16, or with DRB1*0101, DRB1*0103 and DQB1*0501, respectively. Associations with HLA class II alleles have also been shown with allergies to cockroach (Donfack et al. 2000; Kalpaklioglu and Turan 2002) and rat (Jeal et al. 2003). Both of these allergen sources contain important lipocalin allergens. Therefore, it is possible that HLA associations are also found with sensitization to additional lipocalin allergens. In this study, associations between HLA genotypes and sensitization to the major cow allergen Bos d 2 were investigated.

Materials and methods

Subjects

The study included 40 asthmatic farmers whose allergy to cow dander had been confirmed at the Pulmonary Clinic of Kuopio University Hospital. The gender distribution was near to equal (21 female and 19 male). The average age of the subjects was 46.75 ± 1.44 years (mean $\pm$ SEM). All subjects showed a positive response (a decrease in the forced expiratory volume in one second (FEV₁) of >15%) in the specific inhalation challenge with bovine dander allergens (Soluprick SQ, ALK Abelló, Hørsholm, Denmark),

as described previously (Koskela et al. 2003). Additionally, skin prick test (SPT) result of ≥ 3 mm with cow allergen (epithelial preparation from ALK Abelló, Hørsholm, Denmark) at the dilution of 1:100 was required. The sensitization to the major cow allergen was verified by SPT with recombinant (r)Bos d 2, as described previously (Saarelainen et al. 2004). Positive results of ≥ 3 mm were measured at the highest tolerated Bos d 2 concentration which ranged individually from 2 to 200 μ g/ml. All subjects provided signed informed consent for participation, and the study was approved by the Ethics Committee of the Kuopio University Hospital.

The frequencies of HLA alleles and haplotypes of the control subjects originated from a population of 151 consecutive patients waiting for haematopoietic stem cell transplantation (Finnish Red Cross Blood Service, Helsinki, Finland). The control population did not differ in the distribution of the HLA alleles from a group of 99 healthy, Finnish blood donors (unpublished data).

HLA class II genotyping

The HLA-DR/DQ genotypes of the subjects were determined by PCR-SSO method using commercial INNO-LiPA kits (Innogenetics, Gent, Belgium) and some of the samples were further typed

Table 2

Prevalence of HLA haplotypes in Bos d 2-sensitized and control subjects.

Haplotype DRB1*	DQB1*	Bos d 2-sensitized subjects		Control subjects		P value	OR	95% CI
		Number	%	Number	%			
0101	0501	19	23.8	40	13.3	0.025	2.04	1.105–3.767
0102	0501	1	1.3	1	0.3			
0103	0501	0	0.0	1	0.3			
0301	0201	2	2.5	33	10.9	0.017	0.21	0.049–0.891
0401	0301	2	2.5	2	0.7			
0401	0302	9	11.3	23	7.6			
0402	0302	0	0.0	2	0.7			
0403	0302	3	3.8	2	0.7			
0404	0302	12	15.0	16	5.0	0.006	3.15	1.426–6.978
0408	0301	1	1.3	5	1.7			
0701	0303	0	0.0	1	0.3			
0701	0202	1	1.3	15	5.0			
0701	0303	0	0.0	4	1.3			
0801	0402	7	8.8	26	8.6			
0802	0402	0	0.0	1	0.3			
0901	0303	1	1.3	12	4.0			
1001	0501	1	1.3	4	1.3			
1101/04	0301	2	2.5	16	5.3			
1102	0602	0	0.0	1	0.3			
1103	0301	0	0.0	3	1.0			
1201	0301	3	3.8	12	4.0			
1202	0301	0	0.0	1	0.3			
1301	0603	5	6.3	22	7.3			
1301	0303	2	2.5	3	1.0			
1301	0301	1	1.3	1	0.3			
1302	0604	0	0.0	7	2.3			
1302	0606	0	0.0	1	0.3			
1303	0301	0	0.0	3	1.0			
1305	0301	0	0.0	1	1.0			
1401	0503	0	0.0	7	2.3			
1501	0503	0	0.0	1	1.0			
1501	0602	8	10.0	32	10.6			
1501	0609	0	0.0	1	0.3			
1601	0502	0	0.0	2	0.7			
Total		80	100.0	302	100.0			

The percentages involved with statistical significance have been bold.

with PCR-SSP (Olerup™, Genovision, Oslo, Norway). The genotyping was performed in the Clinical Laboratory of the Finnish Red Cross Blood Service (Helsinki, Finland).

Analysis of the allergen peptide binding to HLA class II alleles

The allergen peptide binding to HLA class II molecules was analyzed in more detail by comparing the physical properties of amino acids in the peptide binding clefts of HLA molecules with the properties of anchor amino acids in the peptide epitope. The physical properties of amino acids were obtained from IMGT, the international ImMunoGeneTics information system1 (<http://imgt.cines.fr>) (Pommie et al. 2004), and the peptide binding positions in HLA molecules from the SKDM HLA software (<https://sourceforge.net/projects/skdm>) (Kanterakis et al. 2008).

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (Graphpad Software, San Diego, CA). Fisher's two-tailed exact test was used to analyze the differences between the frequencies of HLA class II alleles and haplotypes in allergic and control subjects. P values of 0.05 or less were considered significant. Odds ratio (OR) values were determined with 95% confidence interval (CI).

Results

According to the skin prick tests, all 40 cow-asthmatic subjects were sensitized to cow and its major allergen Bos d 2. The average

wheel diameters ($\pm$ standard errors of the mean) to cow dander and to Bos d 2 were 5.83 ± 0.43 mm and 5.00 ± 0.25 mm, respectively.

HLA class II genotype associations with sensitization to the major cow allergen Bos d 2

The distributions of DRB1 and DQB1 alleles in Bos d 2-sensitized and control subjects are shown in Table 1. The frequency of the DRB1 alleles 0101 and 0404 was significantly higher in allergic than in control subjects (23.8% vs. 13.2%, $P < 0.05$, OR = 2.0 and 15.0% vs. 5.3%, $P < 0.01$, OR = 3.2, respectively). Also, the DQB1 alleles 0302 and 0501 were significantly over-expressed in the sensitized study population (30.0% vs. 14.2%, $P < 0.01$, OR = 2.6 and 26.3% vs. 15.2%, $P < 0.05$, OR = 2.0, respectively). In contrast, cow-sensitized subjects had a significantly lower frequency of DRB1*0301 and DQB1*0201 alleles than did the control subjects (both 2.5% vs. 10.9%, $P < 0.05$, OR = 0.2).

The results with haplotypes are in line with the associations observed with individual alleles (Table 2). The frequencies of the haplotypes DRB1*0101/DQB1*0501 and DRB1*0404/DQB1*0302 were significantly higher in allergic than in the control subjects. In contrast, a significantly lower frequency of the haplotype DRB1*0301/DQB1*0201 was present in sensitized than in the control subjects.

Peptide binding properties of the predisposing and protective alleles

The comparison of physical properties of peptide binding amino acids reveals differences between the predisposing and the

Table 3

Peptide-binding amino acids within the HLA molecules. The numbers under the pockets indicate amino acid positions in β chain.

DRB1*	Pocket 1			Pocket 4						Pocket 6			Pocket 7					Pocket 9				
	85	89	86	13	71	78	70	74	26	9	11	30	28	61	71	47	67	9	60	57	37	38
0101	V	C	D	F	R	Y	Q	A	L	W	L	C	E	W	R	Y	D	W	Y	D	S	V
0404	V	C	D	H	R	Y	Q	A	F	E	V	Y	D	W	R	Y	D	E	Y	D	Y	V
0301	V	C	D	S	K	Y	Q	R	Y	E	S	Y	D	W	K	F	D	E	Y	D	N	V

Dark grey indicates hydrophobic amino acids. Light grey indicates hydrophilic amino acids. Amino acids H, K, and R are positively charged. Amino acids D and E are negatively charged.

protective HLA alleles (Table 3). In the binding pocket 4, there is a hydrophobic amino acid (A) in the β -chain position 74 of DRB1*0101 and 0404, whereas the β 74 amino acid of DRB1*0301 is hydrophilic (R). There is a hydrophobic amino acid also in the position β 26 of DRB1*0101 (L) and 0404 (F) while the β 26 amino acid is neither hydrophobic nor hydrophilic (Y) in the allele 0301. The situation is similar also in the position β 11 which is located in the peptide binding pocket 6. In the alleles 0101 and 0404, the amino acids in the positions β 37 and β 47 are neutral in hydropathy (S or Y), whereas in DRB1*0301, the amino acids of β 37 and β 47 are hydrophilic (N) and hydrophobic (F), respectively. In addition to hydrophilicity/hydrophobicity, there is a difference in the charge of the amino acids in the position β 74. Alanine (A) of the alleles DRB1*0101 and 0404 is neutral in charge, whereas the amino acid arginine (R) of DRB1*0301 is positively charged.

Discussion

In the present study, we have shown that the frequencies of HLA class II alleles DRB1*0101, DRB1*0404, DQB1*0302, and DQB1*0501 were significantly higher among Bos d 2-sensitized than the control subjects. Due to linkage disequilibrium, DRB1*0101 is known to be inherited with DQB1*0501 and DRB1*0404 with DQB1*0302 (Klitz et al. 2003). As indicated in Table 1, our results suggest that these alleles are significant in increasing the risk for cow allergy and occupational asthma in farmers (see also Table 2).

HLA-DRB1*01 haplotype has been found in the majority of subjects showing T cell reactivity to the immunodominant epitope of mugwort pollen (Jahn-Schmid et al. 2002; Torio et al. 2003). Higher frequencies of DRB1*0101 and DQB1*0501 alleles have also been shown in the Caucasian subjects sensitized to bee venom allergen phospholipase A₂ (Sanchez-Velasco et al. 2005) and cockroach (Donfack et al. 2000) than in non-sensitized controls. An increased risk for cockroach allergy is especially interesting since the cockroach allergen Bla g 4 is also a lipocalin protein (Arruda et al. 1995).

The HLA haplotype DRB1*04-DQB1*0302 has been associated at least with latex allergy (Rihs et al. 2002) and western red cedar-derived asthma (Horne et al. 2000). It has also been suggested that HLA-DRB1*04 would be associated with sensitivity to β -lactoglobulin, a lipocalin allergen of cow's milk (Sakaguchi et al. 2002). However, in contrast to our present results with Bos d 2, our previous data with the major dog allergen Can f 1 suggested that the HLA haplotype DRB1*04-DQB1*0302 would protect from allergy instead of predisposing to it (Kinnunen et al. 2005). This may be explicable by differences observed between the allergenic molecules. We have previously reported that Bos d 2 contains one immunodominant epitope (Zeiler et al. 1999), whereas the T cell epitopes of Can f 1 are distributed into seven regions along the molecule (Immonen et al. 2005).

Our present data indicate that Bos d 2-sensitized subjects express significantly lower frequencies of HLA-DRB1*0301 and DQB1*0201 alleles than the control subjects do. The HLA-DR3 haplotype appears also to be protective in rat allergy (Jeal et al. 2003). It is interesting that cow and rat-allergic subjects share the protective haplotype although the alleles predisposing to the

specific hypersensitivities are different. The HLA-DR3 haplotype was not found to be protective in dog allergy (Kinnunen et al. 2005). However, diminished expression of DR3 haplotype in dog-allergic subjects can not be excluded since subjects carrying DRB1*0301 and DQB1*0201 alleles were missing from the relatively small study population (Kinnunen et al. 2005).

It has been suggested that peptides binding to DRB1*0101 would have a large aromatic or hydrophobic residue (Y, F, W, L, I, V or M) in the binding motif position 1 and a small, noncharged residue (S, T, C, A, P, V, I, L or M) in position 6 (Southwood et al. 1998). We have previously shown that the peptide (p127-142, ELEKYQQLNSERGVN) containing the immunodominant epitope of Bos d 2 tolerates the removal of the first four amino acids without losing its stimulatory capacity for human T cells (Kinnunen et al. 2003). Therefore, the probable core of the epitope begins with Y131 having S in the sixth position (Kinnunen et al. 2003). This is in accordance with our present finding that DRB1*0101 allele is associated with sensitization to the major cow allergen Bos d 2. Interestingly, the immunodominant epitope region of the major horse allergen Equ c 1, to which eight horse-sensitized subjects out of ten showed T cell reactivity, contains two possible epitopes with amino acid I in the first and in the sixth position (Immonen et al. 2007).

Jeal et al. (2003) have previously found some unique amino acids in HLA class II molecules associated either positively or negatively with rat allergy. Similarly to Bos d 2, the major rat allergen belongs to lipocalins which are known to bind hydrophobic ligands. A hydrophobic leucine (L) at position β 30 of HLA-DRB1*07 was suggested to be a plausible allergen binding site in rat allergy. There is a hydrophobic amino acid (C) at β 30 position also in the DRB1*0101 allele which we found to be associated with sensitization to Bos d 2. For cow allergy, β 74 appears to be an even more significant position within the HLA molecules. It is the only position in DRB1*0101, 0404, and 0301 which possesses a hydrophobic amino acid (A) in 0101 and 0404, and a hydrophilic amino acid (R) in 0301 (Kanterakis et al. 2008). In DRB1*0101 and 0404 this unique position β 74 could serve as an important binding position for the hydrophobic anchor amino acid P4 (L) in the immunodominant epitope of the cow allergen. DRB1*0301 with its hydrophilic amino acid at β 74 would not bind the allergen epitope as avidly as DRB1*0101 and 0404 do, and therefore diminish the possibility for allergy. In addition to hydrophobicity, the amino acid at the β 74 position is uncharged (A) in DRB1*0101 and DRB1*0404, and positively charged (R) in DRB1*0301. It is probable that the uncharged P4 amino acid (L) of the allergen will be bound more avidly by an uncharged than by a positively charged amino acid.

Amino acid β 74 is located within the binding pocket P4 of HLA class II molecules (Kanterakis et al. 2008). It has been observed that the P4 pocket is critical for antigen presentation (Stern et al. 1994). It is interesting to note that there are more hydrophobic amino acids in the binding pocket P4 of HLA-DRB1*0101 (three out of six) and 0404 (two out of six) alleles than there are in HLA-DRB1*0301 (none out of six). As mentioned above, hydrophobicity may create more favourable environment for the binding of hydrophobic ligands.

P1, P4, P6, P7, and P9 are known to be important anchor positions for the peptide binding to HLA molecules. It is worth noting that the only hydrophobic or neutral amino acids in the immunodominant peptide core of Bos d 2 are located within positions P1 (Y), P4 (L), P6 (S), and P9 (G). The amino acid within position P7 is hydrophilic, as are those of positions not so important for HLA binding. However, the binding pocket 7 of HLA molecules differs only by one position (β47) in which happens to be the only hydrophobic amino acid in the protective DRB1*0301 allele (F) while that of DRB1*0101 and DRB1*0404 is neutral (Y). A hydrophobic amino acid of DRB1*0301 may reject the hydrophilic amino acid of Bos d 2 more strongly than does the neutral amino acid of the predisposing alleles.

Our present results suggest that the HLA class II alleles DRB1*0101, DRB1*0404, DQB1*0302, and DQB1*0501 are associated with sensitization to the major cow allergen Bos d 2, whereas HLA-DRB1*0301 and DQB1*0201 protect from it. Furthermore, a detailed analysis of the amino acids within these three HLA-DR molecules identified important differences between the susceptible alleles and the protective allele. This study affiliates farming to the occupations in which genetic factors have been shown to associate with allergic sensitization. Previously, HLA associations have been shown for example in health care workers (hevein) (Rihs et al. 2002), sawmill workers (western red cedar) (Horne et al. 2000), laboratory animal workers (rat urine) (Jeal et al. 2003), and bee-keepers (insect venom) (Sanchez-Velasco et al. 2005). Knowing the HLA associations of common sensitizers can facilitate the diagnosis of occupational disorders. Increased knowledge of the risks of allergic diseases may also enhance the development and use of work-related protective materials and safety equipments.

Acknowledgements

The skilful technical assistance of Virpi Fisk, Pirjo Vääntinen and Raija Tukiainen is gratefully acknowledged. This work was financially supported by the Academy of Finland (contract #115160), Kuopio University Hospital (project #5021605) and the foundation of Väinö and Laina Kivi.

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