

HLA class II genotype, HLA-DR B cell surface expression and allergen specific IgE production in atopic and non-atopic members of asthmatic family pedigrees

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Summary

HLA class II polymorphism is variably associated with sensitization to specific allergens, but few convincing HLA associations with asthma or the general state of atopy have been demonstrated. In this study we investigated HLA class II genotype associations with asthma, atopy and specific IgE (sIgE) production to six allergen extracts and six purified major allergens (Der p 1, Der p 2, Fel d 1, Can f 1, Alt a 1 and Phl p 5) in 176 individuals from 20 asthmatic family pedigrees. In selected individuals, cell surface HLA-DR peripheral B-cell expression was correlated with HLA-DRB1 genotype and atopic status. Results showed that HLA-DRB1*08 was negatively associated with asthma (2% vs 17%; $P_c = 0.02$; OR = 0.08) and atopy (0% vs 16%; $P_c = 0.04$; OR = 0.1), while DRB1*15 was positively associated with asthma (36% vs 13%; $P_c = 0.02$; OR = 3.6). Analysis of DRB1 sequences showed that only 29% of individuals with GAG TAC TCT ACG at codons 9–12 in one or both alleles were atopic, compared with 53% of individuals without this sequence ($P = 0.002$; OR = 0.36). DPA1*0201 was negatively associated with sIgE to both grass pollen mix and Phl p 5 (0% vs 23%; $P_c = 0.02$; OR = 0.14). A non-significant trend towards higher HLA-DR B-cell expression was seen in both non-atopic and DRB1*08 individuals. In conclusion, this single centre study has demonstrated a number of HLA class II genotype associations with asthma, atopy and sIgE to grass pollen mix and Phl p 5, including hitherto unreported DRB1*08, DRB1 codon 9–12 and DPA1*0201 associations. No significant associations between HLA-DR expression and DRB1 genotype or atopy were demonstrated, although a trend towards higher expression was seen in non-atopic individuals and individuals of DRB1*08 genotype.

Keywords: HLA, atopy, asthma, sIgE

Introduction

Genetic regulation of the T-cell immune response, mediated via human leucocyte antigen (HLA)-antigenic peptide-T-cell receptor (TCR) molecular interactions, is principally influenced by the extensive polymorphism of the HLA class II genes, although variation in the expressed TCR repertoire may also be important [1]. In addition, it has been suggested that factors which influence the avidity of HLA-peptide-TCR engagement can have important effects on the immune response to antigen [2]. To date, a considerable number of studies have shown that particular HLA class II alleles may

be risk factors for or protective factors against the development of sensitization to specific allergens, especially but not exclusively those of lower molecular weight, although in some cases contradictory results have been obtained from different studies, the bulk of which have been case-control rather than family pedigrees [reviewed in 3]. Conversely, until recently [4], no convincing associations between HLA class II genotypes and asthma had been described, although a number of reports had shown associations between several asthma-associated phenotypes (including house dust mite allergy) and particular HLA class II genotypes [5]. Finally, although the HLA complex on chromosome 6p21.3 has not been linked to the general state of atopy by linkage analysis, one group, using a stricter definition of non-atopic status, has demonstrated a strong association between atopy and

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haplotypes bearing the DRB4 locus, encoding the DR53 serological specificity (DRB1*04, *07 and *09 genotypes) in both family and case-control studies [6].

Aims

In the present study, HLA class II genotypic associations with asthma, atopy and sIgE responses to a panel of six common allergen extracts and six highly purified allergens in 20 asthmatic family pedigrees were investigated. Secondly, HLA class II DR cell surface expression on peripheral B cells from atopic and non-atopic individuals was determined to ascertain whether differences in HLA-DR expression correlate with atopic status and/or HLA-DRB1 genotype. Family pedigrees were selected on the basis of having two generations of asthma with the third generation having childhood asthma or infantile wheeze. This approach was novel in that, for the first time, all principal HLA class II polymorphic loci (DRB1, DQA1, DQB1, DPA1 and DPB1) were analysed by DNA-based PCR-sequence specific oligonucleotide probe (SSOP) typing in a single centre study, combined with an investigation of HLA-DR expression on B cells of atopic and non-atopic individuals.

Subjects and methods

Index families were recruited through the antenatal clinics at Southampton General Hospital if either the father or mother had asthma and in addition, if there were two or three generations of asthma through either the maternal or paternal line. Asthma in the index parents was determined by history and a positive histamine challenge after the post-partum period. All individuals in the extended pedigree were asked to participate by completing a questionnaire (modified from the International Study of Asthma and Allergies in Childhood and International Union Against Tuberculosis and Lung Disease questionnaires). Asthma was defined from questionnaire responses as the presence of recurrent wheezing, or recurrent nocturnal or exercise-induced cough in the last 12 months, which responded to bronchodilator therapy. Subjects had their sensitivity to six common allergen extracts (ALK, Reading, UK) and six purified major allergens (a kind gift of Dr Carsten Schou,

ALK) determined using the Magic Lite system [7]. Samples were scored as positive or negative for the presence of detectable sIgE for each allergen. Allergen extracts included those of house dust mite (*Dermatophagoides pteronyssinus*), cat dander, dog dander, tree pollen mix (equal portions of *Alnus glutinosa*, *Betula verrucosa* and *Corylus avellana*), grass pollen mix (equal portions of *Avena elatior*, *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*) and a mould mix (*Alternaria alternata*, *Aspergillus fumigatus* and *Cladosporium* sp.). Purified allergens consisted of Der p 1 and Der p 2, Fel d 1, Can f 1, Alt a 1 and Phl p 5. DNA was prepared according to standard rapid salt precipitation methods and PCR-SSOP typing performed according to previously published methods, using a panel of 65 digoxigenin-labelled oligonucleotide probes and enhanced chemiluminescent detection of bound probes in a 'dot blot' format [8]. HLA-DR expression on peripheral blood B cells from selected individuals (see Results) was determined using a Becton-Dickinson FACSort and Lysis II software package. Full details of the protocol used are given elsewhere [9]. Briefly, HLA-DR B cell surface expression was analysed for each matched group by measuring the fluorescence of labelling with FITC anti HLA-DR (L243) of CD19⁺ cells (FMC63). Measurements were converted to antibody binding capacity (ABC), which was determined before and after 48 h culture with γ -IFN.

Results

HLA class II genotype associations with asthma, sIgE and atopy

Of 155 individuals entered into the study, 71 had asthma and 56 individuals were atopic. One hundred and thirty-nine individuals were typed for HLA-DRB1, 124 for DQA1, 112 for DQB1, 126 for DPA1 and 103 for DPB1. The frequencies of all genotypes were compared between individuals with and without asthma, atopy (defined as detectable sIgE to any of the six allergen extracts) and sIgE to each of the 12 test allergens, using 2×2 tables and the chi-squared test (ARCUS 1992, version 2.03 A). Probability (*P*) values were corrected for the number of genotypes compared at each locus (corrected $P = P_c$). Results are summarized in Table 1.

Table 1. Summary of HLA class II genotype association with asthma, atopy and presence of specific IgE

Locus	Genotype	Characteristic	Genotype frequency in cases with characteristic (%)	Genotype frequency in controls without characteristic (%)	Chi square P_c value	Odds ratio (uncorr.)
DRB1	*15	Asthma	23/64 (36)	10/75 (13)	0.02	3.6
DRB1	*08	Asthma	1/64 (2)	13/75 (17)	0.02	0.08
DRB1	*08	Atopy	0/49 (0)	14/90 (16)	0.04	0.1
DPA1	*0201	Phl p 5	0/25 (0)	23/101 (23)	0.02	0.14
DPA1	*0201	Grass mix	0/25 (0)	23/101 (23)	0.02	0.14

From this it can be seen that a positive association was found between DRB1*15 and asthma ($P_c = 0.02$, OR = 3.6) and a negative association was seen between DRB1*08 and asthma status ($P_c = 0.02$, OR = 0.08). No significant associations were seen between any of the genotypes and house dust mite, tree pollen, dog dander or mould sensitivity. However, a negative association between DPA1*0201 and grass pollen mix ($P_c = 0.02$, OR = 0.14) was observed. Detectable sIgE to a major allergen component of the grass mix, Phl p 5, was also found to be negatively associated with DPA1*0201 ($P_c = 0.02$, OR = 0.14). No associations between any of the other highly purified allergens and HLA class II alleles at any of the loci examined were found. With respect to atopy, a negative association was seen between non-atopic status and DRB1*08 ($P_c = 0.04$, OR = 0.1). Post-analysis examination of the data revealed a negative association between the presence of the nucleotide sequence GAG TAC TCT ACG at codons 9–12 of the second exon of HLA-DRB1 locus (encoding amino acids Glu, Tyr, Ser, Thr) and atopic status: 29% individuals carrying this motif in one or two alleles were atopic, compared with 53% individuals without this sequence ($P = 0.002$, OR = 0.36).

*B-cell surface expression of HLA-DR on HLA-DRB1*08 atopic and non-atopic individuals*

To investigate possible mechanisms for DRB1*08 associated protection from atopy, expression of HLA-DR on B cells from the following groups of subjects was investigated: (i) non-atopic carriers of HLA-DRB1*08 ($n = 6$); (ii) non-atopic, non-DRB1*08 individuals matched with group (i) individuals for the second DRB1 allele ($n = 5$); (iii) unrelated atopic non-DRB1*08 individuals matched with groups (i) and (ii) for the second DRB1 allele ($n = 6$). Unpaired and paired *t*-tests were used to analyse the mean ABC of the anti-DR antibody of the matched groups and differences of the means of the paired groups after culture with and without γ -IFN. This preliminary investigation showed no significant HLA-DRB1*08 genotype- or atopy-dependent difference in HLA-DR expression, either at baseline or after 48 h culture with and without γ -IFN. The data did, however, suggest a trend for higher HLA-DR expression in the non-atopic (mean L243 ABC on CD19 + ve cells = 174 425) compared with atopic individuals (mean = 147 437). In addition the DRB1*08 genotype group of individuals showed a tendency for higher HLA-DR expression (mean L243 ABC on CD19 + ve cells = 182 930) compared with individuals with non DR8 genotype (mean = 159 213).

Discussion

Firstly, results obtained from this single centre study showed both positive (DRB1*15) and negative (DRB1*08) associations between HLA class II alleles and the asthma phenotype.

DRB1*15 has been previously shown to be associated with ragweed sensitivity in asthmatics, but not with ragweed sensitive subjects who suffered with allergic rhinitis alone [10]. A number of other studies have shown associations between DRB1*15 and ragweed pollen and/or some of its purified allergens [11]. The negative association between DRB1*08 and both asthma and atopy found in this study has not been reported before, including two previous family based studies from the UK, one of which was from this centre, but with a differing study design based on selection of families with HDM allergy [12,13]. Secondly, a comparison of genotype frequencies within the study group did not show any of the previously reported associations with sensitivity to major allergens, as determined by the presence of detectable serum sIgE to the allergen extracts. However, genotypic analysis revealed a negative association between DPA1*0201 and detectable sIgE to a grass pollen mix extract and a highly purified component of the extract, Phl p 5. The only other HLA genotype and allergy study in which the DPA1 locus has been examined found an association with Japanese cedar pollenosis and DPA1*02022 [14]. Therefore, the DP subregion merits further investigation in future HLA studies of atopic disease and sIgE responses. Thirdly, this study does not directly support a previously reported association with between atopy and the DR53 (DRB4) associated DRB1*04, 07 and 09 genotypes in French Caucasian individuals [6] and this is in agreement with the independent results of Holloway *et al.* [13], from our own centre. However, presence of the sequence motif GAG TAC TCT ACG (exon 2 codons 9–12), which is contained within the first hypervariable region of exon 2, encoding part of the base of the peptide binding cleft of the expressed molecule, was negatively associated with atopy. This sequence is not found in any of the DRB1*04, *07 or *09 groups of alleles, but is found in all DRB1*08 alleles, which may provide a unifying hypothesis for these apparently discordant findings. The DRB1*08 negative association with atopy/asthma was not paralleled by a negative DRB1*08 association with any of the sIgE sensitivities (although another study from our centre indicates that DRB1*08 haplotypes predispose to peanut allergy [15]) and this may suggest that the DRB1*08 group of alleles does not specifically influence unique allergen/MHC interactions. An alternative explanation of these findings is that there may be a more global aberration in antigenic peptide presentation to T cells associated with DRB1*08 bearing haplotypes. The DRB1*08 group of alleles has a number of unusual features which would support this hypothesis. They are expressed by a single DRB locus (DRB1) on the class II haplotype on which they occur, unlike the majority of the other DRB1 alleles which are associated with one of three other expressed DRB loci (DRB3[DR52 expressing], DRB4[DR53] or DRB5[DR51]).

Thus there is less diversity of HLA class II DR molecules expressed on antigen presenting cells in carriers of the DRB1*08 alleles, with implications for antigen presentation and for thymic selection of the T-cell repertoire. In addition, DRB1*08 has been reported to have a much lower level of transcription than other DRB1 alleles, i.e. sevenfold less than DRB1*04 [16], which may result in a significantly lower surface expression of DRB1*08. This could significantly affect the antigen exposure threshold for activation and the development of T- and B-cell responses in HLA-DRB1*08 individuals, while the intensity of T cell receptor engagement could determine the cytokine expression pattern (Th1 vs Th2) of allergen specific helper T cells [2]. Therefore, fourthly, we sought to determine whether cell surface HLA-DR expression was related to HLA-DRB1*08 and/or atopic status. Peripheral B cells were chosen for this analysis, which is therefore preliminary rather than definitive. However, results did not reveal any HLA-DRB1*08 or atopy-associated differences in HLA-DR expression. In fact, countering our original hypothesis, a non-significant trend towards increased HLA-DR expression was seen in both DRB1*08 and non-atopic individuals. There are many limitations to this study design, but the influence of HLA class II genotype-cell surface expression and Th1 vs Th2 responses requires definitive investigation.

Conclusions

This single centre study of multicase asthma families revealed a number of associations with asthma, atopy and production to specific allergens, including a hitherto unreported DRB1*08 associated protection from atopy and asthma and DPA1*0201 protection from sIgE production to Phl p5 and grass pollen mix, although findings from other studies were not replicated. The DRB1*08 group of alleles contains a functional exon 2 sequence motif, shared among other alleles, which was also negatively associated with atopy. This sequence motif is not shared with other alleles (DRB1*04, 07 and 09) which have previously been reported as associated with atopy, providing a possible common motif which may protect against atopy. Therefore our results support a role for HLA class II polymorphism in influencing allergen-specific and non-specific IgE production. Studies of HLA-DR expression on B cells did not support an initial hypothesis that DRB1*08 may protect against atopy via association with lower levels of HLA-DR expression in DRB1*08 individuals. Rather, a trend towards higher HLA-DR expression was seen in non-atopic individuals and individuals of DRB1*08 genotype, which requires confirmation in more extensive studies.

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