

Evidence for a role of HLA DRB1 alleles in the control of IgE levels, strengthened by interacting TCR A/D marker alleles

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Summary

Background MHC class II alleles at human chromosome 6p21.1 and alleles in the TCR A/D locus at human chromosome 14q11.2 have been implicated in susceptibility to specific allergies and the modulation of total serum IgE. It has also been hypothesized that HLA and TCR allelic interactions may have a strong influence on predisposition to allergic disease.

Objective This study was performed to investigate the influence of HLA-DRB and DQB1 alleles and D14S50 alleles (adjacent to TCR A/D locus on 14q11.2), individually and in combination, on total serum IgE levels, and on the development of specific allergies.

Methods We performed an association study between HLA-DRB, HLA-DQB1 polymorphisms, D14S50 alleles, total serum IgE expression and specific allergies to house dust mite, grass pollens and cat fur. A sample of 181 individuals was drawn from a larger set of 2415 adults, sampled at random from a district in Nottingham.

Results Strong association was observed between HLA-DRB1*0701 allele and high total serum IgE expression ($P < 0.001$). D14S50 alleles alone showed no evidence for independent association. However, there was a significant interaction between DRB1*0701 and D14S50 allele 170 such that, when both were present, there was a further increase in total serum IgE levels.

Conclusion This study suggests that DRB1*0701 allele is involved in the modulation of total serum IgE, and that there is an interaction between DRB1*0701 and a marker adjacent to TCR A/D in the control of IgE expression.

Keywords: IgE, HLA, MHC class II, TCR, HDM, specific allergies.

Clinical and Experimental Allergy, Vol. 30, pp. 1371–1378. Submitted 13 April 1999; revised 28 March 2000; accepted 17 April 2000.

Introduction

Atopy is a syndrome characterized by exaggerated and persistent IgE responses to environmental allergens. Atopy involves several components including total and allergen-specific serum IgE titres and skin prick test reactivities to allergens. Studies of the genetics of atopy have focused in part on the HLA MHC class II genes, which is pertinent in view of their role in antigen presentation to the immune system [1].

Different HLA genes may represent risk factors for, or protective factors against the development of allergic disease [2,3]. Numerous studies have investigated the role of class II genes in restriction of the IgE responses to a variety of allergens. The strongest and most consistently reported association is between ragweed allergen (*Ambrosia artemisiifolia*) sensitivity and DRB1*1501 (DR2) [4–6]. A number of studies have examined the role of MHC class II alleles in predisposition to the general atopic state. In a large French family of three generations comprising 35 individuals, susceptibility to allergy, with or without asthma-related symptoms, was frequently associated with HLA class II DR4 and DR7 specificities [7]. Another study compared

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MHC class II allele frequencies in 56 unrelated atopic patients (defined by either positive skin test reactivity to one or more of 17 common allergens, raised total serum IgE, or bronchial hyper-responsiveness) and their frequency in 39 healthy controls with no familial or personal history of asthma or atopy. DR4 (DRB1*04) and DR7 (DRB1*07) alleles were present in 39.2% of the patients, but only in 2.5% of the controls, and were reported to increase susceptibility to the general allergic state rather than to specific responses to allergens [8]. Conversely, DQA1*0103 and DQB1*0502 alleles were significantly higher in the control group. Similar findings were also observed in another subset of 44 Caucasian subjects from Eastern France with allergic asthma and allergy to birch pollen and fruits, compared to 39 healthy Caucasian controls. In these DR4 and DR7 allele frequencies were significantly higher in atopics as compared to controls [9]. Although such significant findings have been observed in three separate sets of subjects by Aron *et al.* to our knowledge, they have not been confirmed by an independent study.

The T-cell receptor (TCR) genes are also candidates for influencing the predisposition to allergic diseases. Genetic linkage has been reported between markers close to the TCR A/D locus on human chromosome 14q11.2 (FCA.TA1 and D14S50) and allergic responses to common allergens and total serum IgE [10]. In the same study, testing for linkage with TCR B marker on chromosome 7q was negative. Other studies have reported linkage of the TCR A/D locus to total serum IgE [11], and to asthma [12]. Interestingly, the latter study also reported asthma linkage to markers close to the HLA region on 6p21. In a set of 53 multiplex asthma families recruited from Southampton (UK), we observed linkage between the FCA.TA1 microsatellite and atopy (defined as positive skin reactivity to one or more common allergens) [13]. In the latter study, we observed no allelic association between the TCRA and either total serum IgE or skin reactivity to allergens. However we found an association between the 170 bp allele of an adjacent microsatellite (D14S50) and total serum IgE [14]. Similar results were also obtained by Moffatt *et al.* [15]. Therefore, the chromosome 14q11.2 region may contain a gene(s) predisposing to allergic disease.

Testing for simultaneous association between reactivity to known environmental antigens and both TCR A/D and HLA MHC class II alleles suggested potential interaction between these loci in restricting the response to such antigens. The TCR AV8S1 polymorphism (AV8S1*2) was reported to be strongly associated with allergy to Der p II, a major antigenic component of *Dermatophagoides pteronyssinus*. In the same subjects, allergy to Der p II was also found to be associated with DRB1*1501. Reactivity to Der p II was confined to subjects who were positive for TCR AV8S1*2 and HLA DRB1*1501, demonstrating germline

HLA DR and TCR AV interaction in restricting the response to exogenous antigen [16].

In this study we have explored three areas. Firstly, we have tested for association between HLA-DRB and DQB1 alleles and total serum IgE and/or specific skin reactivity to common allergens. Secondly, we have tested for association between microsatellite D14S50 alleles (which maps adjacent to the TCR A/D locus) and total serum IgE and/or specific reactivity to common allergens. Finally, we have looked for evidence of interaction between HLA DR/DQ and D14S50 alleles on the predisposition to allergic disease.

Materials and methods

Subjects

Subjects were recruited in an epidemiological study of the aetiology of asthma and chronic obstructive pulmonary disease, as previously described [17], and comprised a random sample of 2633 adults aged 18–70 years from the electoral register of a Local Authority Area of Nottingham. Information on respiratory symptoms was collected by questionnaire, and measurements made of one second forced expiratory volume (FEV₁) and forced vital capacity (FVC) by spirometry (Vitalograph, Buckingham UK). Skin reactivity was determined to three common allergens in the UK, namely, *D. pteronyssinus*, grass pollen and cat fur (Bencard, UK). Measurements of airway reactivity to methacholine [18] were obtained in 2415 individuals, from whom data was available for IgE levels, and blood available for DNA extraction in 2296. Bronchial hyperreactivity was defined as a PD₂₀ FEV₁ of 12.25 µmol or less, and was positive in 314 individuals (13%) of the sample. Subjects who displayed PD₂₀ FEV₁ of >12.25 µmol were labelled as nonhyperreactive. A clinician confirmed diagnosis of asthma was reported by 191 individuals (8%). Atopy was defined as the presence of any positive allergen skin test of 3 mm or more larger than the saline control to *D. pteronyssinus*, cat fur or grass pollens, which was positive in 1056 subjects (44%). Total serum IgE was measured using radio-immunoassay.

A subsample of 720 individuals was identified for genetic analysis. DNA extraction was then successfully performed for 560 individuals of whom a subsample of 181 individuals was identified for this study. This subsample was selected such that asthma was over-represented, by taking all those with doctor diagnosed asthma and bronchial hyperreactivity (*n* = 89), and a second group, selected at random from those who had no reported asthma, no hyperreactivity to methacholine, and were nonatopic (*n* = 92). The whole sample of 181 subjects was then examined as a single sample for association with HLA and D14S50 alleles.

DNA typing for HLA-DRB & DQB1

All of the 181 individuals were genotyped at the HLA DRB and DQB1 loci using the PCR sequence-specific primer (SSP) method [19]. This technique has a high degree of resolution, with each primer pair defining two linked, cis-located polymorphic sites, which facilitates the typing of heterozygous individual [20]. All individuals included in the study were typed for 47 specific alleles or groups of alleles within the HLA DR and DQ specificities. Amplification primer sets for DRB and DQB1 alleles were purchased from the Oxford Transplant Centre, Nuffield Department of Surgery, Churchill Hospital, Oxford, UK. The design of these primers was based on published nucleotide sequences or sequences available from Genbank accession codes and was given by Bunce *et al.* [20]. All amplification primers were designed to give a melting temperature between 58 °C and 62 °C. Amplification control primers giving rise to a 796-bp fragment from the third intron of HLA DRB1 were included in all PCRs as a positive amplification control. PCRs were performed in 192 well microtitre plates. PCR mixtures consisted of 67 mM Tris-HCl, pH 8.8, 16.6 mM ammonium sulphate, 2 mM MgCl₂, 0.01% (v/v) Tween 20, 200 µM of each dNTP, 1–4 µM of each allele-specific primer, 0.1 µM of DRB1 control primers, 100 ng of DNA and 0.1875 U Taq DNA Polymerase (Promega). The final volume of all PCR reactions was 13 µL not including a 10-µL mineral oil overlay. Eight µL of the buffer, DNA and enzyme mixture was added to 5 µL of primer mix with a multichannel pipette. PCR amplification was performed using a MJ Research PTC-200 Thermal Cycler. The PCR cycles were as follows; 1 min at 96 °C followed by five cycles of 25 s at 96 °C, 45 s at 70 °C, 45 s at 72 °C followed by 21 cycles of 25 s at 96 °C, 50 s at 65 °C, 45 s at 72 °C followed by four cycles of 25 s at 96 °C, 60 s at 55 °C, 120 s at 72 °C. PCR plates were sealed with Thermowell™ sealer (Costar) and dipped in mineral oil (to improve the plate to block contact).

PCR products were electrophoresed in 1.0% agarose gels containing 0.5 µg/mL ethidium bromide after the addition of 5 µL of loading buffer, consisting of 0.25% w/v Orange G, 30% v/v glycerol, and 0.5× TBE buffer (89 mM Tris-Base, 89 mM boric acid, 2 mM EDTA, pH 8.0). The gels were run for 15 min (or until the Orange G had travelled 3 cm) at 15 V/cm in 0.5× TBE buffer and visualized using UV illumination. Accurate scoring of HLA alleles was confirmed by two independent investigators.

The D14S50 marker primer set was supplied by the MRC HGMP Resource Centre [21]. All individuals in the data set were genotyped for this marker using an ABI Prism 377. PCR reactions were performed in 96-well microtitre plates in 10 µL volume containing: 40 ng of DNA, 1.5 mM MgCl₂, 1.0 µL of 10× Thermo buffer, 150 µM of each dNTP, 9.4 pM

of each primer, and 0.1 units of Taq DNA polymerase (Promega). The PCR cycles were as follows: 95 °C for 5 min, then 26 cycles of 55.4 °C for 20 s, 72 °C for 20 s, and 90 °C for 30 s, followed by a final extension period at 72 °C for 10 min. To ensure the accuracy of the typing and minimize variation in allele sizing between gels, a control PCR product from one individual was incorporated in all the electrophoresis runs. Runs in which the control DNA produced inconsistent allele sizes were repeated. Genotyping was successful in 177 individuals. DRB and DQB1 alleles encountered in this sample and their frequencies are presented in Table 1a, whilst D14S50 allelic classes are described in Table 2.

Statistical methods

Data analysis was performed using the SPSS (6.0) statistical package (SPSS 6.0 for VAX/VMS, SPSS Inc., USA).

The dependent variables (clinical phenotypes) in our association analyses were total IgE, atopy (any allergen skin test positivity) and skin test positivity to HDM (*D. pteronyssinus*), grass pollen and cat fur. Total IgE was log transformed to normality for analysis. In primary analysis, we assessed the association of DRB1*04, DRB1*07 and DQB1*05, as well as allele 170 of D14S50 with these clinical outcomes. In secondary analysis, we looked for associations with all other alleles on DRB, DQB1 and D14S50, and tested interactions between the effects of HLA and TCR A marker alleles on total IgE.

Student *t*-tests were used initially to test associations with total IgE, and adjustment for age, sex and smoking status was performed by multiple linear regression. Multiple linear regression was also used to test the significance of interactions [22]. Associations with skin test reactivity were analysed by chi-squared test, and multiple logistic regression.

A total of 39 comparisons were required to examine for associations with DRB, DQB1 and D14S50. To maintain an overall significance level of 0.05, the Bonferroni correction for the number of comparisons performed requires a significance level of $P = 0.0013$ for each allele test.

Sample size determination was based on standard formulae and this study had 90% power to detect a 1 and a 0.75 SD difference in IgE for alleles with a frequency of 5% and 10%, respectively [25].

Results

The age of the 181 subjects at the time of ascertainment ranged between 18 and 69 years. Of these, there were 105 subjects who have never smoked, 40 ex-smokers, and 36 subjects who were current smokers at the time of the assessment. Seventy-seven individuals (42.5%) were atopic

and 89 (49.2%) were hyperreactive to methacholine ($PD_{20} < 12.25$), all of whom also reported doctor diagnosed asthma as predetermined by our selection criteria. Log IgE in this cohort ranged between 0.00 and 3.4.

Linkage disequilibrium analysis

Association between HLA alleles (which would reflect the phenomenon of linkage disequilibrium and the presence of extended HLA haplotypes) was examined using chi-square

tests. The associations observed are consistent with the previously described haplotype DQB1*02, DQB1*03032, DRB4*01, DRB1*04, DRB1*07 and haplotype DQB1*05, DRB1*0101/4, DRB1*16, respectively [8,23,26,27].

Association analysis with HLA-DRB & DQB1

The results for the association analysis between HLA-DRB and DQB1 alleles and log IgE and atopy are given in Table 1a. Allele DRB1*0701 showed strong association

Table 1a. The results of association analysis between DRB, DQB1 alleles and log IgE and atopy (*P*-values from *t*-tests and chi-squared tests, respectively)

	DRB and DQB1	Log IgE			Atopy		
		Allele freq. (%)	Mean log IgE (SD) in allele +/- subjects	<i>P</i> -value	Atopic (74) in allele (+) (%)	Atopic (102) in allele (-) (%)	<i>P</i> -value
1	DRB1*0101/4	30 (17.0)	1.40 (0.74) / 1.62 (0.86)	NS	7 (23.3)	23 (76.7)	0.02
2	DRB1*0103	2 (1.1)	1.12 (0.91) / 1.59 (0.85)	NS	1 (50.0)	1 (50.0)	NS
3	DRB1*0301	68 (38.6)	1.54 (0.89) / 1.61 (0.82)	NS	27 (39.7)	41 (60.2)	NS
4	DRB1*0401-22	56 (31.8)	1.48 (0.82) / 1.63 (0.86)	NS	23 (41.0)	33 (59.9)	NS
5	DRB1*0701	46 (26.1)	1.98 (0.72) / 1.43 (0.85)	<0.001	21 (45.7)	25 (54.3)	NS
6	DRB1*0801-11	11 (6.3)	1.87 (0.81) / 1.56 (0.85)	NS	7 (63.6)	4 (36.4)	NS
7	DRB1*0901	3 (1.7)	0.96 (0.87) / 1.60 (0.84)	NS	3 (100)	0 (00.0)	NS
8	DRB1*1001	2 (1.1)	1.60 (0.42) / 1.58 (0.85)	NS	1 (50.0)	1 (50.0)	NS
9	DRB1*1101-15	24 (13.6)	1.69 (0.85) / 1.57 (0.85)	NS	10 (41.7)	14 (58.3)	NS
10	DRB1*1201-3	9 (5.1)	1.67 (0.88) / 1.58 (0.85)	NS	6 (66.7)	3 (33.3)	NS
11	DRB1*1301	16 (9.1)	1.27 (0.76) / 1.61 (0.85)	NS	7 (43.8)	9 (56.2)	NS
12	DRB1*1302	11 (6.3)	1.35 (1.00) / 1.60 (0.84)	NS	4 (36.4)	7 (63.6)	NS
13	DRB1*1303	6 (3.4)	1.62 (0.53) / 1.58 (0.86)	NS	3 (50.0)	3 (50.0)	NS
14	DRB1*1401/7	4 (2.2)	1.24 (0.17) / 1.59 (0.85)	NS	2 (50.0)	2 (50.0)	NS
15	DRB1*1501/3-5	50 (28.4)	1.75 (0.83) / 1.52 (0.85)	NS	25 (50.0)	25 (50.0)	NS
16	DRB1*1502	1 (0.6)	2.93 (0.00) / 1.57 (0.84)	NS	1 (100)	0 (00.0)	NS
17	DRB1*1601-6	6 (3.4)	0.89 (1.00) / 1.61 (0.83)	0.04	2 (33.3)	4 (66.7)	NS
18	DRB3*0101	70 (39.8)	1.53 (0.87) / 1.62 (0.83)	NS	29 (41.4)	41 (58.6)	NS
19	DRB3*0201	2 (1.1)	0.70 (0.99) / 1.59 (0.84)	NS	0 (00.0)	2 (100)	NS
20	DRB3*0202	50 (28.4)	1.55 (0.84) / 1.59 (0.84)	NS	23 (46.0)	27 (54.0)	NS
21	DRB3*0301	10 (5.7)	1.48 (0.95) / 1.59 (0.84)	NS	4 (40.0)	6 (60.0)	NS
22	DRB1*01 (E)	84 (46.4)	1.65 (0.85) / 1.52 (0.84)	NS	37 (44.0)	47 (56.0)	NS
23	DRB1*01 (NE)	18 (10.2)	1.89 (0.70) / 1.55 (0.86)	NS	6 (33.3)	12 (66.7)	NS
24	DRB5*01/02	57 (32.4)	1.69 (0.85) / 1.54 (0.82)	NS	28 (49.1)	29 (50.9)	NS
25	DQB1*02	92 (52.3)	1.63 (0.86) / 1.53 (0.83)	NS	40 (43.5)	52 (56.5)	NS
26	DQB1*0301/4	69 (39.2)	1.64 (0.85) / 1.55 (0.84)	NS	32 (46.4)	37 (53.6)	NS
27	DQB1*0302	24 (13.3)	1.33 (0.65) / 1.62 (0.87)	NS	8 (33.3)	16 (66.7)	NS
28	DQB1*03032	16 (9.1)	1.97 (0.66) / 1.54 (0.85)	0.05	5 (31.3)	11 (68.7)	NS
29	DQB1*04	10 (5.7)	1.97 (0.82) / 1.56 (0.84)	NS	7 (70.0)	3 (30.0)	NS
30	DQB1*05	41 (23.3)	1.29 (0.76) / 1.67 (0.85)	0.01	11 (26.8)	30 (73.2)	0.02
31	DQB1*0601	3 (1.7)	1.57 (1.48) / 1.58 (0.84)	NS	2 (66.7)	1 (33.3)	NS
32	DQB1*0602	45 (25.6)	1.75 (0.82) / 1.52 (0.85)	NS	23 (51.1)	22 (48.9)	NS
33	DQB1*0603	19 (10.8)	1.42 (0.80) / 1.60 (0.85)	NS	9 (47.4)	10 (52.6)	NS
34	DQB1*0604-9	8 (4.5)	1.10 (1.05) / 1.60 (0.83)	NS	2 (33.3)	6 (66.7)	NS

Table 1b. The influence of the DRB1*0701 and DQB1*05 on log IgE stratified by atopic (skin test positivity) status

The influence of DRB1*0701 on log IgE stratified by atopy				
	DRB1*0701	Number	Mean log IgE (SD)	<i>P</i> -value interaction
Atopy (+)	(+)	21	2.52 (0.59)	0.8
	(−)	52	2.07 (0.71)	
Atopy (−)	(+)	25	1.52 (0.44)	
	(−)	77	1.02 (0.64)	
The influence of DQB1*05 on log IgE stratified by atopy				
	DQB1*05	Number	Mean log IgE (SD)	<i>P</i> -value interaction
Atopy (+)	(+)	11	1.91 (0.72)	0.4
	(−)	62	2.25 (0.70)	
Atopy (−)	(+)	30	1.06 (0.64)	
	(−)	72	1.17 (0.63)	

The influence of the DRB1*0701, and DQB1*05 on log IgE stratified by atopic (skin test positivity) status. SD: standard deviation, SE: standard error, category (+) for present, and category (-) for absent, freq. frequency, DRB1*01 (E): for the expressed allele, DRB1*01 (NE) for the nonexpressed allele. Please note that provided *P*-values are not adjusted for multiple comparisons. NS indicates nonsignificance at the 5% level. To account for multiple comparisons (39 tests with all HLA and D14S50 alleles) a *P*-value of 0.0013 or less is taken to indicate significance. Only DRB1*0701 showed significance at this level.

with total IgE ($P < 0.001$) but not with atopy. Allele DQB1*05 was negatively associated with total IgE ($P = 0.01$) and with atopy ($P = 0.02$), neither of these associations were significant at the $P = 0.0013$ level. Other HLA alleles, which showed association with DRB1*0701 showed weaker (DQB1*03032) or no association (DRB4*01, DRB1*04, DQB1*02 and DRB1*1601-6) with total IgE, or

atopy. In multiple linear regression, the effects of DRB1*0701 and DQB1*05 were stronger than, and independent, of any of the remaining interacting alleles in the control of IgE levels. The effect of DRB1*0701 on total IgE is similar in those with and without atopy (Table 1b).

We observed lower DQB1*05 frequency in atopics which is nonsignificant when corrected for multiple comparisons

Table 2 Association analyses of D14S50 allelic classes and log IgE and atopy (*P*-values from *t*-tests and chi-square tests, respectively)

Allele	Log IgE			Atopy		
	Frequency (%) <i>n</i> = 348	Mean log IgE (SD) in allele $\pm$ subjects	<i>P</i> -value	Positive (76) Allele + (%)	Negative (101) Allele - (%)	<i>P</i> -value
168	97 (0.27)	1.66 (0.84)/1.58 (0.88)	NS	42 (43.3)	55 (56.7)	NS
170	46 (0.13)	1.63 (0.91)/1.62 (0.84)	NS	20 (43.5)	26 (56.5)	NS
172	76 (0.22)	1.55 (0.85)/1.68 (0.87)	NS	33 (43.4)	43 (56.6)	NS
174	82 (0.24)	1.68 (0.85)/1.57 (0.87)	NS	36 (43.9)	46 (56.1)	NS
Rare-alleles	46 (0.13)	1.60 (0.94)/1.63 (0.83)	NS	20 (43.5)	26 (56.5)	NS

Alleles with frequency of less than 10% were pooled together and analysed under the category of rare alleles. *n*: number, NS: nonsignificant at 5% level.

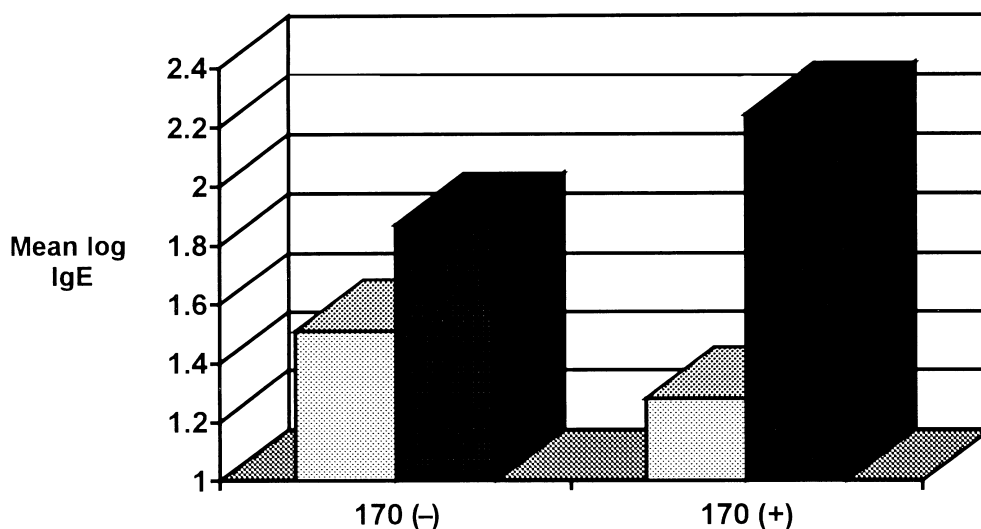


Fig. 1. Illustration of the effect of D14S50 allele 170 and DRB1*0701 interaction on log transformed IgE. Hatched area, DRB1*0701 (-) and black area, DRB1*0701 (+).

(data not shown). A similar trend was observed in individuals with grass pollen allergy (11.4% compared to 27.3% in controls), and with HDM and cat fur allergies (17.0% in the affected group vs 26.0% in the nonaffected groups for HDM allergy, and 16.7% vs 26.2% for cat allergy, respectively).

Association analysis with D14S50 alleles

D14S50 microsatellite typing identified 10 alleles of which six were seen in less than 10% of the subjects. Alleles with less than 10% frequency were pooled together and analysed as one category of rare alleles [24], resulting in a reduction of the total number of allele classes analysed to five.

Table 2 details the results of the association analysis of individual D14S50 alleles with log IgE and atopy. No evidence of association was observed between the alleles analysed and either log IgE or atopy. D14S50 alleles also showed no evidence for association with skin reactivity to HDM, cat fur, or grass pollens (data not shown).

Modification of total IgE by interacting DRB1*0701 and D14S50 alleles

In a multiple regression model on log IgE, incorporating DRB1*0701, allele 170 and an interaction term between the two alleles, the interaction was significant ($P = 0.05$). The difference in mean log IgE between DRB1*0701(+) and DRB1*0701(-) subjects who were positive for allele 170 was significantly higher (0.96) than the difference in the 170(-) group (0.36) (Fig. 1).

Discussion

This study presents evidence of strong association between total serum IgE levels and the DRB1*0701 allele, which is strengthened by interaction of allele 170 of D14S50 (adjacent to TCR A/D on 14q11.2).

In contrast to our study, Aron *et al.* reported association of atopy (defined as raised total IgE, positive skin reactivity to allergens, or bronchial hyperreactivity) with both DRB1*04 and DRB1*07 [7,8]. We observed evidence for linkage disequilibrium between the two alleles, but only DRB1*0701 showed strong association with total serum IgE. Further multiple regression analysis also excluded other alleles in linkage disequilibrium with DRB1*0701, from being independent factors in the control of total serum IgE. The DRB1*0701 association was also independent of skin test reactivity to common allergens, as shown by the observation of similar increase in mean log IgE in DRB1*0701(+) subjects irrespective of their atopy status.

DQB1*0502 and the DQA1*01, DQB1*0501, DRB1*01 haplotype were reported to confer significant protection against isocyanate-induced asthma [28,29], and atopy [7,8]. In our study, we observed a trend of lower mean total serum IgE, and lower incidence of skin test reactivity to three common allergens in DQB1*05 (+) subjects. The association of DQB1*05 to log IgE in our study loses significance when corrected for the number of alleles examined, and caution is therefore required when interpreting this result. However, collectively, the latter studies suggest that DQB1*05 may have a protective influence against the development of the atopic status in general. This contrasts with the effect of DRB1*0701 which seems to primarily modulate total serum IgE.

Genetic linkage has been previously reported between the TCR A/D locus and IgE responses to major allergens [10], total serum IgE [11], atopy [13] and asthma [12]. We also observed an association of allele 170 bp of D14S50 (on 14q11.2) to total serum IgE and skin reactivity based atopy definition in 53 multiplex asthmatic families from Southampton, UK [14]. D14S50 allele 170 (corresponding to allele 3) was also reported to be associated with grass allergy [30]. In the present study, D14S50 alleles did not showed an independent association with different measures of atopy, but allele 170 showed significant positive interaction with DRB1*0701 in modulating log IgE. Therefore, allele 170 may be in linkage disequilibrium with a gene/s in the 14q11.2 which interact with DRB1*0701 to influence total IgE levels. Interestingly, Moffatt *et al.* also reported association between D14S50 allele 3 (which correspond to 170 bp) and increased IgE titres to the timothy grass pollen *Phleum pratense* in DRB1*07 positive subjects [30]. Although many candidate genes which map to 14q11.2 may account for this association, the TCR A/D locus is probably the likely candidate. HLA/TCR interaction in the modification of the immune response has been reported in both animal and human studies. In the B6 mouse, germline variation in particular TCR AV segments has been shown to have a major influence on MHC restriction [31,32]. In mice, selections of CD4+ or CD8+ T-cell subsets were determined by complementarity-determining region 1 (CDR1) or CDR2 preferential interactions with class I and class II MHC molecules. In humans, one study has provided evidence of a modifying influence on an individual's response to a foreign antigen, through interaction between a germline element in the TCR AV region and a particular HLA-DRB1 type [16]. Future study of interaction between specific polymorphisms within the TCR A/D and HLA alleles in relation to the allergic diathesis is required to confirm this hypothesis.

In conclusion, this study confirms the previously reported strong association between DRB1*0701 (but not DRB1*04) and the allergic diathesis, and presents evidence for allelic interaction between DRB1*0701 and a marker close to the TCR A/D locus which modulates the level of total serum IgE.

Acknowledgements

We would like to thank all the volunteers for their generous participation in this study, as well as A. Knox, I. Parvord, K. Richards, A. Wisniewski, A. Tattersfield and S. Weiss who were involved in the collection of the clinical data and M. Plunkett in manuscript preparation. This study was supported by grants from the MRC, National Asthma Campaign, British Lung Foundation, and Northern and Yorkshire Regional Health Authority.

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