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Germline *TCR-A* restriction of immunoglobulin E responses to allergen

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Abstract Immunoglobulin E responses to known environmental antigens (allergens) may serve as a general model to investigate germline genetic restriction of the immune response. We have previously shown genetic linkage between IgE responses to major allergens and the T-cell receptor (*TCR*) *A/D* locus, but not to *TCR-B*, implying that elements in *TCR A/D* restrict the ability to react to specific antigens. We now show, in two sets of subjects from the same population, a strong allelic association between a *VA8.1* polymorphism (*VA8.1*2*) and reactivity to *Der p* II, a major antigenic component of the house dust mite *Dermatophagoides pteronyssinus*. Association was also seen between *Der p* II IgE titres and *HLA-DRB1*1501* alleles. Reactivity to *Der p* II was confined to subjects who were positive for *VA8.1*2* and *HLA-DRB1*1501*, demonstrating germline *HLA-DR* and *TCR-A* interaction in restricting the response to exogenous antigen.

Introduction

Atopy (familial asthma, rhinitis, and eczema) is characterized by immunoglobulin E (IgE) responses to soluble antigens known as allergens. The development of disease in atopic individuals depends on the type of allergens to which they react (Cookson et al. 1991; Sears et al. 1989). Atopic asthma, which now affects one child in seven in the United Kingdom (Strachan et al. 1994), is strongly influenced by house dust mite (HDM) allergy (Cookson et al. 1991; Peat et al. 1987; Sears et al. 1989), whereas grass pollen sensitization carries much less risk of asthma. It is therefore of clinical interest to investigate the genetic control of the specific IgE response. Inhaled allergen sources such as HDM or grass pollens are complex mixtures of proteins. A number of major allergens, to which IgE responses are consistently found, have been identified from each allergen source. In the case of HDM, the two most important allergens are *Der p* I (254 000 M_r) and *Der p* II (141 000 M_r), each of which seems to have four major B-cell epitopes (Chapman et al. 1987; Lind et al. 1988). Peptide mapping of *Der p* II has shown that T-cell clones from different individuals may also react to common T-cell epitopes (O'Hehir et al. 1993).

The *HLA* and T-cell receptor (*TCR*) genes are candidates for germline influences on specific allergen responses. The association of *HLA* haplotypes and ragweed allergy was the first human Ir (immune response) gene to be recognized (Levine et al. 1972), and *HLA-DR* restriction of IgE reactions to allergen is well documented (Marsh et al. 1981; Young et al. 1994). However, the *HLA* genes on their own do not account for the differences in an individual's IgE reactions to allergen (Young et al. 1994). The *TCR* repertoire is not random, and is influenced by germline (genomic) polymorphism (Gulwani-Akolkar et al. 1991; Loveridge et al. 1991; Moss et al. 1993). We therefore examined the *TCR* loci for genomic restriction of IgE responses.

We have previously established linkage of specific IgE responses to the *TCR-A/D* locus on chromosome 14 (but not to *TCR-B*) in two sets of subjects (Moffatt et al. 1994). The *TCR-A/D* region is complex (Arden et al. 1995), and contains many elements which might influence specific antigen recognition. Localization of these elements depends on associations between specific alleles and IgE responses. We have not found associations with IgE responses and alleles of the *TCR-A* microsatellites FCA.TA1 and D14S50 (Moffatt et al. 1994). However, it has been previously demonstrated that *VA8* may be in excess in T-cell clones reacting to HDM (Wedderburn et al. 1993), and so we have now investigated a bi-allelic polymorphism in *VA8.1* (Cornélis et al. 1993) for association with IgE titres to HDM and its major antigens. In order to investigate possible interactions between *HLA* and *TCR* loci, the subjects were also *HLA-DR* typed.

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Table 1 Association between IgE titres to house dust mite antigens and *VA8.1* polymorphism

Panel A subjects					
Der p I		n	Mean (SE)	t	*P*
VA8.1 allele	1	316	1.133 (0.092)	−1.91	0.057
	2	490	1.367 (0.081)		
Der p II		n	Mean (SE)	t	*P*
VA8.1 allele	1	316	0.620 (0.056)	−2.78	0.006
	2	490	0.833 (0.052)		
Panel B subjects					
Der p II		n	Mean (SE)	t	*P*
VA8.1 allele	1	337	0.374 (0.044)	−2.17	0.030
	2	493	0.507 (0.042)		
Combined subjects					
Der p II		n	Mean (SE)	t	*P*
VA8.1 allele	1	653	0.493 (0.036)	−3.57	0.000
	2	983	0.669 (0.034)		

Statistical analysis of allergen responses is demanding if loci with many alleles, such as the *HLA-DR* system, are to be studied. Multiple regressions, which can disentangle the effects of interacting variables, and which reduce multiple comparisons to a single test of significance, have been used successfully in the past to elucidate *HLA* associations with allergens (Farewell and Dahlberg 1984; Young et al. 1994), and were used in the present study.

Materials and methods

Four-hundred-and-ten subjects from 88 nuclear families (Panel A) were sub-selected from an Australian general population sample of 1020 subjects in 230 families who have been extensively studied for asthma and atopy (Hill et al. 1995; Moffatt et al. 1994). The mean age of the subjects was 24.2 yrs (SD 14.4), and 52% were male. An additional 426 subjects from the same population sample (Hill et al. 1995) were also studied (Panel B). Their mean age was 27.6 yrs (SD 14.3) and 52% were male. Sera were tested for elevation of IgE titres to the major allergens *Der p* I and *Der p* II (from the house dust mite *D. pteronyssinus*) by chemiluminescence assay using monoclonal anti-IgE (ALK Laboratories, Hørsholm, Denmark). Results were converted to RAST classes.

Typing of the *VA8.1* segment polymorphism for Panel A subjects was by electrophoresis of specific polymerase chain reaction (PCR) products on 10% acrylamide gels as described previously, with a number of modifications (Cornélis et al. 1993). For the Leader PCR, 200 ng of genomic DNA was used as template with 0.5 μM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl (pH 8.8 at 25 °C), 50 mM KCl, 1% Triton X-100 and 1.5 mM $MgCl_2$ and 0.75 units of *Taq* DNA polymerase in a final reaction volume of 25 μl. Amplification conditions were 95 °C for 5 min followed by 30 cycles of 95 °C for 1 min, 55 °C for 45 s and 72 °C for 1 min. For the Intronic PCR, 1 μl of a 1/100 dilution of the Leader PCR product was used as template with substitution of the V5′ primer for the leader primer in the PCR. Intronic PCR conditions were the same as for the Leader PCR, except that the anneal temperature was increased to 56 °C and the cycle number was decreased to 14. PCR products were run on 10% polyacrylamide gels (19:1 polyacrylamide: bis-acrylamide, 10% glycerol, 0.5 × TBE) for 22 h at 4 °C, and visualized by silver staining. Known controls were included on each gel. Since the nucleotide change results in the introduction of an *Ava* II restriction site, genotyping of Panel B subjects was by restriction digestion of the Intronic PCR product. Following Intronic amplification, 5 μl of PCR product was digested with 5 units of *Ava* II (New England Biolabs, Beverly, MA) at 37 °C for 1 h. Resultant products were analyzed on 2% agarose gels. *VA8.1* allele 1 was identified by a single 264 base pair (bp) band and allele 2 by 122 and 142 bp bands.

To ensure conformity of results for the two methods of typing for *VA8.1*, 30 subjects genotyped by single structured conformational polymorphism (SSCP) were also typed by the *Ava* II restriction digest protocol. Eight of these subjects were also sequenced. Sequenced controls of each genotype were included in each set of digests performed.

*HLA-DRB1** typing was by sequence-specific oligonucleotide probing with digoxigenin-ddUTP (Boehringer Mannheim UK, Lewes, East Sussex) labeled probes (Wordsworth et al. 1990). *HLA-DR* types determined included *HLA-DRB1*01–*16*. Subtypes were recognized for *HLA-DRB1*02* (*HLA-DRB1*1501,* and *HLA-DRB1*1502,*1601,* and **1602*), for *HLA-DRB1*05* (*HLA-DRB1*11* and *HLA-DRB1*12*), and for *HLA-DRB1*06* (*HLA-DRB1*13* and *HLA-DRB1*14*).

Genotypes for *VA8.1* and *HLA-DRB1* were checked independently by two individuals who were blind to the phenotype. Failed amplifications were repeated twice. In Panel A, amplification of *VA8.1* failed in one subject and amplification of HLA-DRB1 failed in two subjects. Both amplifications failed in six subjects. In Panel B, *VA8.1* did not amplify in eight subjects, *HLA-DRB1* did not amplify in two subjects, and neither locus amplified in three subjects. Overall, only five of the failed amplifications had positive serology for *Der p* I or II.

The results were analyzed by SPSS Version 4.1 for VAX/VMS. Associations of IgE titres to *Der p* I and *Der p* II with *VA8.1* alleles were tested by t-tests. Similar results were found with non-parametric Mann-Whitney tests. Exact Odds Ratios were calculated from contingency tables by the STATXACT program (Cytel Corp, Cambridge, MA). The effect of familial correlation on the results was examined by use of the ASSOC routine of the SAGE program (Release 22, Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland).

Table 2 Interaction between *TCR-VA8.1*, *HLA-DRB1**, and IgE titres to *Der p* II. Multiple regression analysis

Panel A subjects			
Variable	B (Standard error)	t	*P*
VA8.1	0.352 (0.118)	2.98	0.0030
*HLA-DRB1*1501*	0.235 (0.080)	2.92	0.0035
Constant	−0.027 (0.202)	−0.13	0.89
Not significant: *HLA-DRB1 *01 *03 *04 *07 *11*			
Panel B subjects			
Variable	B (Standard error)	t	*P*
*HLA-DRB1*1501*	0.256 (0.088)	2.91	0.0038
VA8.1	0.164 (0.064)	2.56	0.0106
Constant	−0.107 (0.160)	−0.67	0.50
Not significant: *HLA-DRB1 *01 *03 *04 *07 *11*			
Combined subjects			
Variable	B (Standard error)	t	*P*
*HLA-DRB1*1501*	0.286 (0.073)	3.90	0.0001
VA8.1	0.201 (0.052)	3.90	0.0001
Constant	−0.052 (0.130)	−0.41	0.68
Not significant: *HLA-DRB1 *01 *03 *04 *07 *11*			

Multiple forward stepwise regressions were carried out (SPSS 4.1) with the IgE titre in RAST classes to *Der p* II as the dependent variable. Independent variables were *VA8.1* (allele 1 = 1, allele 2 = 2), and the six most common *HLA-DRB1* types; **01, *15, *03, *04, *07,* and **11* (absent = 1, present = 2). Backward stepwise regressions gave the same results.

Results

In the Panel A subjects, allele 2 of the *VA8.1* polymorphism (*VA8.1*2*) showed a significant association with higher IgE titres to *Der p* II [($P = 0.006$) (Table 1)]: a weak association was seen to *Der p* I ($P = 0.057$). The association with *VA8.1*2* was confirmed in the Panel B subjects [($P = 0.03$)(Table 1)], and was highly significant in the pooled subjects [($P = 0.000$)(Table 1)]. The IgE titres were approximately 25% higher in subjects with *VA8.1*2*, in both groups and in the combined data.

In order to account for possible interacting effects with *HLA-DRB1*, multiple regression analysis was carried out with IgE titre to *Der p* II as the dependent variable, and *VA8.1* and the six most common *HLA-DR* types as independent variables. The results showed that *HLA-DRB1*1501* was positively associated with IgE titres to *Der p* II in Panel A and Panel B subjects, and in the pooled data (Table 2). In the pooled data, the mean *Der p* II IgE titre when *VA8.1*2* and *HLA-DRB1*1501* were together in the same subject was 1.14 ± 0.14 RAST classes, compared with 0.56 ± 0.025 when neither allele was present. This level of enhanced IgE response is likely to be of clinical significance.

To test further for interaction between *HLA-DR* and *VA8.1* the combined subjects were partitioned into *HLA-DRB1*1501*-positive and -negative groups, and the *Der p* II titres were categorized into positive (≥2 RAST classes) and negative responses (<2 RAST classes). In the *HLA-DRB1*1501*-negative subjects, the association between *Der p* II and *VA8.1*2* was marginal (odds ratio 1.32, 95% confidence interval (CI) 0.99–1.75, $P = 0.053$), whereas in the *HLA-DRB1*1501*-positive subjects the association was highly significant (OR 2.80, 95%CI 1.55–5.06, $P = 0.0006$), confirming the presence of the *TCR-A/HLA-DR* interaction.

As part of wider investigations into the genetics of asthma, we recruited and studied families rather than individuals. While association between phenotypes and alleles may be tested in families, familial aggregation of the trait under study means that individuals in a family may not be fully independent (George and Elston 1987). We therefore examined the results with the ASSOC program, which includes familial correlation in tests of association between quantitative traits and genotypes (Elston et al. 1994; George and Elston 1987). Using this program, the χ^2 for the association between *Der p* II titres and *VA8.1* and *HLA-DRB1*1501* genotypes in the combined subjects was 7.64 with 1df ($P = 0.0057$), indicating that the positive results of the study were not due to chance aggregation of familial effects.

Discussion

The findings of this study are indirectly supported by other investigations. *VA8* has been found to be dominant in T-cell clones reacting with HDM (Wedderburn et al. 1993). Polymorphisms in *TCR-VB* segments are associated with differential levels of usage of the segments in the peripheral repertoire (Vissinga et al. 1994), and it is possible that the *VA8.1* polymorphism modifies the expression or affinity of

this segment. An effect of *HLA* genotype on the peripheral *VB* repertoire is recognized (Akolkar et al. 1993), and it has been suggested that the *VA* repertoire is influenced by exposure to particular antigen (Gulwani-Akolkar et al. 1995).

In the B6 mouse, germline variation in particular *TCR-VA* segments has been shown to have a major influence on MHC restriction (Sim et al. 1996), consistent with our finding of *HLA/TCRA* interaction in human subjects. In the mouse, selection of CD4 or CD8 T-cell subsets was determined by complimentarity-determining region 1 (CDR1) or CDR2 preferential interaction with class I and class II MHC molecules. The human *VA8.1* polymorphism alters a framework residue, with an unknown effect on the *VA8.1* CDRs (Cornélis et al. 1993). More detailed genetic and functional studies are therefore necessary to determine whether the *VA8.1* polymorphism itself or an element in linkage disequilibrium is responsible for our observed results.

In summary, our study shows that a germline element in the *TCR-VA* region interacts with a particular *HLA-DR* type to modify the response to foreign antigen. Genetic linkage of antigen responses is not seen to the *TCRB* locus (Moffatt et al. 1994), so that restriction of response may be a particular feature of *TCRA/D*. The results have implications for understanding the genetics of susceptibility to infectious and autoimmune diseases. In contrast, however, to autoimmune diseases, which are rare, and in which the offending antigens are often unknown or poorly defined, atopy, defined by positive skin tests to common allergens, affects between one-third and one-half of many Western populations (Cline and Burrows 1989; Holford-Strevens et al. 1984;. Peat et al. 1987), and many major allergens are well characterized at the DNA and protein level. Specific allergy may therefore serve as a model system for understanding the immunogenetics of the humoral response to specific antigen.

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