

Atopy, respiratory function and HLA-DR in Aboriginal Australians

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The Class II genes of the MHC represent a major locus with quantified effects on atopic (allergic) phenotypes in many studies of westernized Caucasians. Although asthma is considered a disease of western societies, typical components of the asthma phenotype, such as elevations of the IgE, are seen with parasitic infestation. We have therefore investigated the effects of the HLA-DRB1 locus on asthma and its intermediate phenotypes in Aboriginal people from the Kimberly region of Australia who were suffering from endemic hookworm infection. Recognizable correlates of allergic asthma were present in the subjects, including skin test positivity to house dust mite (HDM), specific IgE responses to HDM, and the total serum IgE. HLA-DRB1 alleles did not predict the presence of asthma, but multi-allelic tests of association showed the locus accounted for ~33% of the variance of the total serum IgE concentration and 17% of the variance of the specific IgE titres to HDM. Genetic admixture was excluded as a cause of the results. These effects of the MHC on IgE levels were an order of magnitude greater than that seen in Caucasians, consistent with the hypothesis that the genetic predisposition to allergic disease may be driven by adaptation to helminth infection. The results further suggest that parasitism *per se* is not protective against asthma.

INTRODUCTION

Allergic (atopic) asthma has increased in prevalence through the last century and is now the most common chronic disease of childhood (1). Ninety per cent of children with asthma show signs of IgE-mediated allergy to common inhaled proteins, known as allergens. Asthma is associated most closely with allergy to house dust mite (HDM) and to a lesser degree with allergy to cat dander and moulds (2).

Atopic disease is most common in countries with a high standard of living (3,4), suggesting that the increase in the prevalence of allergic disease is due to environmental factors associated with the lifestyle of developed societies. Much attention has therefore been given to the hypotheses that atopic disease results from an absence of infection (5), and that this

operates through failure to suppress an innate bias to Th2 immunity (6). IgE-mediated inflammation is a key component of the immune response to helminth infection, and examination of children with parasitic disease supports alternative hypotheses that polyclonal IgE may protect against mast cell degranulation (7,8) or that parasite-induced IL-10 suppresses allergic disease (9).

Asthma and its associated phenotypes are made up of approximately equal genetic and environmental components in Caucasians who are free of recurrent parasitic or other infections (10). It is therefore of interest to examine the genetic contribution to these phenotypes in populations exposed to recurrent parasitic infections.

A number of genome-wide searches for linkage to asthma and its associated traits have been carried out and show

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reproducible patterns of linkage (11,12). The major histocompatibility complex (MHC) region on chromosome 6 has shown consistent linkage to asthma-associated phenotypes in several studies (13–16) and may therefore be considered to be a major locus influencing allergic diseases. Studies of HLA Class II alleles have established that the locus influences specific IgE responses (17,18) as well as total serum IgE levels (19). Approximately 4% of the variation in IgE against specific allergens and 3% of the total serum IgE concentration is accounted for by the HLA-DR locus in Caucasians (19).

In the present study we have investigated the HLA-DRB1 genes as a model to examine the determinants of allergic responses in the skin and the lung in an isolated population of Australian Aborigines. At the time of the study, these individuals suffered from a high level of endemic helminthic parasitization: population surveys had indicated that 77% of the population and 93% of children aged 5–14 were infected with hookworm at any one time (20). In addition, 30% of the children would be expected to have *Giardia duodenalis* infection; 20% *Hymenolepis nana* infection, and 13% infection with *Entamoeba coli* (21). These high levels of infection have been successfully treated since our study. The study forms part of a wider study of the determinants of respiratory function and disease in this community that is aimed at identifying reversible and preventable factors accounting for a high morbidity and mortality in Aboriginal Australians.

RESULTS

The subjects were between 4 and 83 years of age. There were 108 males and 126 females. Twenty-eight subjects (12%) reported a non-Aboriginal parent or grandparent. Thirty-three subjects (14.1%) had been told by their doctor that they had asthma, and 96 (41%) currently smoked cigarettes.

The mean adult (>17 years) forced vital capacity (FVC) was 3.82 l (SD ± 0.76) in males and 2.75 l (± 0.67) in females, and the forced expiratory volume in 1 s (FEV₁) was 3.16 ± 0.75 l in males and 2.26 ± 0.66 l in females. These results are 20–30% lower than for Caucasian Australians of similar stature, as has been previously observed in this and other Aboriginal populations (22,23). One-hundred and eighty-one subjects successfully completed the protocols for measuring bronchial responsiveness: of these, 52 subjects (21.6%) were classified as having bronchial hyper-responsiveness (BHR). The IgE was log normally distributed with a geometric mean level of 1998.2 IU/l (range 10.0–42,192.6 IU/l).

Although 65% of the subjects had a positive RAST to HDM and 43% had a positive RAST to grass pollen, only 6% had a positive prick skin test to HDM and only 4% had a skin reaction to grass pollen. Skin tests to HDM correlated with specific IgE levels to the same allergen ($r = 0.388$, $P < 0.001$) but not to serum IgE levels ($r = 0.15$, $P = 0.82$). Multiple regression analysis with positive skin tests to HDM as the dependent variable showed that the presence of positive prick skin tests was positively correlated with specific IgE responses to HDM; however, a high total serum IgE concentration negatively predicted positive skin tests (Table 1). Similar results were seen when analysing skin and specific IgE responses to grass pollen (Table 2). These findings are different

Table 1. Regression analyses of effects on end organs. Dependent variable: skin test house dust mite (multiple regression)

Variable	<i>B</i> (±SE)	<i>T</i>	<i>P</i>	<i>R</i> ² (variable)
RAST HDM	0.790 (0.108)	7.28	0.0000	0.141
ln (IgE)	−0.371 (0.097)	−3.82	0.0002	0.054
Constant	2.873 (0.672)	4.276	0.0000	

Variables not in the equation: age, sex, smoking, admixture.

Table 2. Regression analysis of effects on end organs. Dependent variable: skin test grass pollen (multiple regression)

Variable	<i>B</i> (±SE)	<i>T</i>	<i>P</i>	<i>R</i> ² (variable)
RAST grass pollen	0.487 (0.074)	6.59	0.0000	0.116
ln (IgE)	−0.223 (0.058)	−3.84	0.0002	0.056
Constant	1.662 (0.429)	3.88	0.0001	

Variables not in the equation: age, sex, smoking, admixture.

from those observed in Caucasians, where correlations between skin tests, RAST responses and the total serum IgE are all positive (2,24).

Thirty-three subjects (15%) had doctor-diagnosed asthma. The presence of asthma was predicted by the presence of positive skin tests to HDM, as well as by the presence of BHR (Table 3).

Twenty different alleles of the HLA-DRB1 locus were recognized (Table 4). Alleles previously observed in Kimberly Aboriginal people (HLA-DRB1*1502, *0405, *0412, *08032, *1408 and *1409) accounted for 74% of alleles in these subjects. Potentially Caucasian alleles (HLA-DRB*03, *07 and *13) accounted for 6.2%. One-way analyses of variance detected no significant effects of the locus on asthma ($P = 0.98$) or BHR ($P = 0.68$).

Multi-allelic tests of association showed positive effects of the locus on the total serum IgE concentration and specific IgE titres to HDM (Table 5). No effect on IgE titres to grass pollen was observed, and lung volumes and BHR also showed no significant association with the locus.

Examination of specific alleles showed that HLA-DRB1*03, *07 and *13 were negatively associated with the total serum IgE, and that *08032 was positively associated with this trait (Table 6). Specific IgE responses to HDM allergen were negatively associated with *07 [$P = 0.0005$, variance (σ^2) = 6.5%] and positively associated with *08032 ($P = 0.0004$, $\sigma^2 = 8.2\%$).

A formal test for genetic admixture (25), in which the within-family variance of a trait is compared with the between-family variance showed no influence of admixture on any of the traits studied. Inclusion of recognized Caucasian parents or grandparents as a covariate did not change any of the significant associations with the locus. Even after omission of potentially Caucasian alleles (HLA-DRB*03, *07 and *13) the total serum IgE was still significantly associated with the locus (multi-allelic test $P = 0.0067$, $\sigma^2 = 11.3\%$) as was the RAST response to HDM (multi-allelic test $P = 0.0003$, $\sigma^2 = 16.3\%$).

Table 3. Regression analysis of effects on end organs. Dependent variable: asthma (logistic regression)

Variable	<i>B</i> ($\pm$ SE)	Wald (d.f.)	<i>P</i>	Exp (<i>B</i>)
Skin test HDM	1.277 (0.510)	6.26 (1)	0.0123	3.585
Bronchial hyper-responsiveness	1.745 (0.501)	12.13 (1)	0.0005	0.175
Constant	-0.742 (0.955)	0.60 (1)	0.44	

Variables not in the equation: age, sex, admixture, ln (IgE), RAST HDM, smoking.

The dominant allele for positive association to HDM remained HLA-DRB*08032 ($P = 0.0046$, $\sigma^2 = 5.9\%$).

DISCUSSION

The results show an HLA-DR effect that accounts for 33% of the variation in the total serum IgE concentration and 17% of the specific IgE to HDM. This is in marked contrast to an HLA-DR effect in non-Aboriginal Australians that accounts for only 4% of the variation in total serum IgE concentration and 3% of the variation in the serum IgE titres specific to HDM (19).

Although we did not measure IgE titres against parasites, previous surveys have indicated a very high level of infection with hookworm (20), and frequent infections with *Giardia duodenalis*, *Hymenolepis nana* and *Entamoeba coli* (21). It is therefore likely that the very high total serum IgE concentrations seen in these subjects were the result of helminth infestation.

The results show the importance of the locus to serum IgE concentrations in the presence of endemic helminthic infection. The high IgE levels may represent a typical response to this type of infection, regardless of the underlying genetic background, or might result from evolutionary pressures to evolve polymorphic IgE responses against parasitic infections. In the latter case, it is possible that the presence of such pressures may have resulted in heightened genetic susceptibility to allergic disease in other tropical and sub-tropical regions. Progressive Westernization of the Third World might therefore be anticipated to bring an increasing burden of asthma and other atopic diseases.

The study has also shown that atopic phenotypes, such as asthma and positive skin tests to common allergens, may still be identified in the presence of endemic parasitic infection. The proportion of subjects who had positive specific serum IgE titres to HDM and grass pollens were higher than in many Caucasian populations, but was in marked contrast to the proportion of subjects with positive skin test responses to the same allergens. The multiple regression analysis has shown that this discrepancy may be attributed to a negative association between high IgE concentrations and positive skin tests to allergens. The use of positive controls indicates that there were no systematic difficulties in reading skin tests in these subjects. This negative association is consistent with the hypothesis that humans possess a limited number of mast-cell binding sites which become saturated by polyclonal IgE in the presence of parasitism (7). Alternatively, unknown factors arising from chronic infection might have systematically raised IgE concentrations and suppressed mast cell numbers or activity.

Table 4. HLA-DRB1 allele frequencies

HLA-DRB1 allele	Frequency (%), all subjects	Frequency (%), unrelateds
*01	9 (1.9)	5 (2.9)
*1502	61 (13.2)	17 (9.9)
*03 ^a	15 (3.2)	9 (5.2)
*04	38 (8.2)	13 (7.6)
*04w15 (*0405)	14 (3.0)	4 (2.3)
*0412	27 (5.8)	11 (6.4)
*07 ^a	9 (1.9)	9 (5.2)
*08032	149 (32.3)	49 (28.5)
*09	6 (1.3)	3 (1.7)
*11	1 (0.2)	0 (0.0)
*12	14 (3.0)	4 (2.3)
*13 ^a	5 (1.1)	5 (2.9)
*1401	7 (1.5)	3 (1.7)
*1402	11 (2.4)	3 (1.7)
*1404	1 (0.2)	1 (0.6)
*1407	1 (0.2)	1 (0.6)
*1408	32 (6.9)	15 (8.7)
*1409	60 (13.0)	18 (10.5)
*1410	1 (0.2)	1 (0.6)
*0103 (DRBR)	1 (0.2)	1 (0.6)

^aSignifies potentially Caucasian alleles.

It is notable that the HLA-DR genotypes correlated with IgE responses to HDM, but not to grass pollens. HLA-DR association with IgE responses to grass pollen allergens is inconsistent or absent in Caucasians (19,26), perhaps due to failure of the HLA-DR alleles to restrict IgE responses against grass pollen epitopes. It will be of interest to investigate the effect of HLA-DR alleles on parasite-specific IgE.

Only a small proportion of the total serum IgE is made up of IgE specific to known allergens (10) or to parasite antigens (27). Although in our subjects IgE specific to HDM and the total serum IgE concentration associated with the same alleles, this is not the case in Caucasians (19). Further work is therefore desirable to investigate if factors elsewhere within the MHC and in linkage disequilibrium with HLA-DR alleles result in non-specific enhancement of IgE production.

There is recognized genetic admixture in the population that we studied, with 12% of the subjects reporting a non-Aboriginal parent or grandparent. However, including admixture as a covariate did not alter any of the significant correlations or associations found by the study. Transmission tests for admixture were negative and exclusion of potentially non-Aboriginal HLA-DRB1 alleles did not negate the strong associations seen between the locus and measurements of total and specific IgE. The findings are therefore unlikely to be the result of unrecognized population stratification.

Despite our observation that a protective effect of high IgE levels could be observed on skin tests, elevations of the IgE and skin and serum reactions to HDM were still associated with the presence of asthma in these subjects. The community receive regular care from an experienced doctor, and similar prevalence of asthma has been observed in other Aboriginal Australians (28), so the findings may not be attributed to inaccurate classification. The results therefore suggest that parasitism *per se* is not protective against asthma.

Table 5. Multi-allelic tests of association with HLA-DRB1

Phenotype	Covariates	χ^2 (d.f.)	<i>P</i>	Variance due to marker
ln (IgE)	—	47.22 (6)	0.0000	27.1%
	Age, smoking	56.6 (6)	0.0000	34.6%
	Age, smoking, RAST HDM	44.87 (6)	0.0000	27.1%
	Age, smoking, admixture	54.37 (6)	0.0000	33.2%
RAST HDM	—	19.97 (6)	0.0028	10.8%
	Smoking	32.24 (6)	0.0000	17.9%
	Smoking, admixture	31.42 (6)	0.0000	17.3%

Table 6. Association of individual alleles with the total serum IgE

Phenotype	Allele	Covariates	χ^2 (d.f.)	<i>P</i>	Direction of association	Variance due to allele
ln (IgE)	HLA-DRB1*03	Age, smoking	14.94 (1)	0.0001	Negative	15.5%
	HLA-DRB1*07	Age, smoking	35.55 (1)	0.0000	Negative	21.1%
	HLA-DRB1*13	Age, smoking	11.18 (1)	0.0008	Negative	7.3%
	HLA-DRB1*08032	Age, smoking	10.06 (1)	0.0015	Positive	12.7%

Uncorrected *P*-values are shown: however, alleles that did not give a *P* < 0.05 after a Bonferroni correction of 33 are not included in the table.

MATERIAL AND METHODS

Study population

An isolated tropical Aboriginal community in the coastal Kimberley region of north-west Western Australia was surveyed in April 1993. The community receives medical care from the Royal Flying Doctor Service, and supervision of Public Health from the Health Department of Western Australia. All individuals over the age of 4 years took part in the survey. A total of 234 subjects were recruited, of whom 171 were from 19 inter-related pedigrees and 63 were unrelated individuals.

Pedigrees were constructed on the basis of histories given by the subjects, and inheritance checked by genotyping of all subjects with HLA and other polymorphic genetic markers (29). The 19 pedigrees studied comprised five two-generation families and 14 three-generation families. On average, families contained nine members (range = 2–37). On average, each father had 2.0 offspring and each mother 2.8 offspring. Multiple paternity was present within nine sibships.

Personal or parental informed consent was obtained from all subjects with the assistance of local community health workers who were employed in the project. This study was approved by the Human Rights Committee of the University of Western Australia and by the Council of the Aboriginal Community.

Data collection

Individual and family histories of respiratory symptoms, smoking and doctor-diagnosed asthma were assessed at interview using a modified British Medical Research Council questionnaire (MRC 1965). Questionnaires relating to children

were administered to a parent (usually the mother). Modifications to the questionnaire were made with the aid of the public health physician (R.S.) by translation of questions into the local idiom as required. Stated age was verified from community health records and census data. Ancestry was defined as 'Aboriginal' if there were no known non-Aboriginal ancestors. 'Admixture' was defined as one or more non-Aboriginal parents or grandparents. 'Smoking' in subjects was defined as current cigarette smoking ≥ 1 cigarette daily for >1 year assessed by questionnaire.

The FVC and the FEV₁ were measured with a dry bellows spirometer (Vitalograph Ltd, Buckinghamshire UK). The best of three technically satisfactory tests was recorded according to ATS recommendations.

Skin prick testing to *Dermatophagoides pteronyssinus* (HDM), mixed grass pollen, cat and dog dander, *Aspergillus fumigatus*, *Alternaria alternata* and a negative control (Dome-Hollister-Steir, Spokane, USA) was carried out. Wheal diameters were calculated minus the negative control. Bronchial responsiveness to methacholine was measured (30,31); the maximum dose administered was 12 μ mol. The dose to provoke a 20% fall in the FEV₁ (PD₂₀) was estimated by linear interpolation. BHR was defined as a PD₂₀ less than or equal to 4 μ mol methacholine (28,31). Blood was taken by venipuncture for IgE assays, eosinophil and total white cell counts, and DNA studies.

IgE assays

Measurement of total IgE was undertaken using the Pharmacia FEIA CAP system (Pharmacia Diagnostics Sweden). Standard controls were included in the assays. Owing to the presence of very high titres in most of the samples, sera for the

determination of total IgE were diluted 1 in 5 and assayed, giving a range of 10–10 000 kU/l. Twenty sera were further diluted 1 in 20 and re-assayed. Specific IgE was measured by the same system, and a positive test was defined as >1 RAST unit.

Genotyping

DNA was extracted from whole blood samples by standard phenol–chloroform techniques. HLA-DRB1* typing was by SSO probing with digoxigenin-ddUTP (Boehringer Mannheim, Germany) labelled probes as described previously (32,33). Additional typing of Aboriginal alleles was performed using 11th Histocompatibility Workshop protocols and SSO probes (34,35). Genotypes were checked independently by two individuals who were blind to the phenotype.

Correlations between variables were examined by Spearman and Pearson correlation coefficients and by multivariate and logistic regression (SPSS 6.1). The total serum IgE was log_e transformed before analyses. The presence of asthma was coded as 1 = no, 2 = yes, BHR was coded as 1 = no, 2 = yes, smoking was coded as 1 = yes, 2 = no, and admixture was coded as 0 = no 1 = yes.

Association of all alleles with doctor-diagnosed asthma was sought by one-way analysis of variance (SPSS 6.1). Allelic associations with quantitative traits were examined by the QTDI program, which allowed variance-components testing of family-based samples for association, admixture and transmission disequilibrium (25). To avoid multiple comparisons, multi-allelic tests were initially performed, and individual alleles were only examined if the multi-allelic tests were positive.

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