

Histocompatibility antigens in asthma: population and family studies

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ABSTRACT One hundred and twenty-two patients with intrinsic asthma, extrinsic atopic asthma, or asthma with allergic bronchopulmonary aspergillosis were tissue typed for the HLA A, B, and C loci. No associations were found with any of the clinical groups, or with serum total IgE concentrations. Sixty-eight members of 10 families where more than one member was affected by asthma were studied. The segregation of haplotypes in siblings of the *propositi* who were or were not affected by asthma did not differ from the predicted segregation, and there were no differences when atopy or serum total IgE were considered. No biologically important association between HLA and asthma has been shown.

The possibility of increasing understanding of the mechanisms by which inherited factors influence disease has stimulated the study of human leukocyte antigens (HLA). HLA is controlled by genes within the major histocompatibility complex, which is associated with other aspects of immune response, some complement components, and susceptibility to certain diseases (Svejgaard *et al*, 1975).

Asthma is a multifactorial disease, and the influence of genetic factors is not understood. Environmental factors interact with atopy, which itself is influenced genetically (Pepys, 1973; Marsh *et al*, 1974). The pattern of inheritance of asthma may be explained best on a multigenic basis (Sibbald and Turner-Warwick, 1979).

The results of previous studies on HLA in atopy and asthma are conflicting. Weak associations with various antigens have been reported in atopic dermatitis (Weck, 1977) and a haplotype association was found in a family study (Scholz *et al*, 1977). An increase in HLA B8 was suggested by Thorsby *et al* (1971) but not confirmed by Rachelefsky *et al* (1976). Other antigens have also been implicated (Brostoff, 1976). The problems of case selection may have contributed to the conflicting results. Interest was created by reports of HLA associations within families with hypersensitivity to purified ragweed antigens (Levine *et al*, 1972; Blumenthal *et al*, 1974), but these

results have been disputed (Black *et al*, 1976). It has also been suggested that HLA antigens may be associated with IgE-mediated sensitivity to specific ragweed antigens (Marsh *et al*, 1973).

We have sought HLA associations in carefully defined populations with intrinsic asthma, extrinsic atopic asthma, and allergic bronchopulmonary aspergillosis, and haplotype associations with asthma and atopy in family studies.

Methods

POPULATION STUDIES

Selection—White patients attending the Brompton Hospital with asthma, defined as airways obstruction varying either spontaneously or with treatment over short periods, were selected. Those satisfying the MRC criteria for chronic bronchitis were excluded. The groups were defined by the following criteria:

Intrinsic asthma—41 patients: (a) no history of allergic factors provoking asthma, (b) no history of allergic rhinitis or eczema, (c) no positive reactions to skin prick testing with 23 common allergens.

Extrinsic atopic asthma—40 patients: (a) definite history of asthma provoked by allergic factors, (b) at least three positive reactions to skin prick testing with 23 common allergens.

Allergic bronchopulmonary aspergillosis (ABPA)—41 patients: (a) positive prick skin test

to *Aspergillus fumigatus* antigen, Beneard 5% w/v (only those with at least three positive skin prick tests to 23 common allergens were selected so that the group is comparable to the extrinsic atopic group with regard to degree of atopy), (b) demonstration of circulating precipitating antibody to *A. fumigatus*, (c) radiographic abnormality of one of three types—transient pulmonary infiltrates, fixed pulmonary shadows, or bronchiectasis.

Many of the ABPA patients had further supporting evidence for the diagnosis—for example, positive five-hour reactions to aspergillus skin testing or positive sputum culture of *Aspergillus* species. Ten had bronchiectasis. The population has been described (Malo *et al.*, 1977).

CLINICAL ASSESSMENT

Patients completed a standard clinic questionnaire of details of their respiratory symptoms and past history and were examined. Prick skin testing was done with the following allergens: *Alternaria*, *A. fumigatus*, *A. terreus*, *Cladosporium herbarum*, *Meruleus lacrimans*, sporobolomyces, *Candida albicans*, yeast, grass, shrub, trees, milk, egg, wheat, fish, nuts, cat, dog, feathers, horse, ascaris, house dust, and *Dermatophagoides farinae*. The diameter of the weal at ten minutes was measured and reactions were considered positive if they measured more than 2 mm providing the control was negative.

A standard 6 ft posteroanterior chest radiograph was taken. Peak expiratory flow rates, forced vital capacity, and forced expired volume in one second were measured by Wright peak flow meter and Vitalograph before and after inhalation of salbutamol by aerosol. Venous blood was taken for serum separation and HLA typing.

Details of sex, age at assessment, age at onset of asthma, presence of allergic asthma-provoking factors, allergic rhinitis or eczema or both, results of skin prick testing, and radiographic abnormality for the three groups are shown in table 1.

FAMILY STUDIES

Sixty-eight members of white families were investigated. The families were selected if the index case (propositus) had at least one other sibling with asthma who was accessible for investigation. As many other first-degree relatives as possible were assessed fully as described above. The other family members completed the asthma questionnaire; were skin prick tested to house dust mite, grass pollen, cat fur, dog fur, *A. fumigatus*, and control; and recorded peak flow measurements before and after inhaled salbutamol.

Table 1 *Asthma population studies—details of subgroups*

<i>Asthma type</i>	<i>Intrinsic</i>	<i>Extrinsic</i>	<i>ABPA</i>
No in group	(41)	(40)	(41)
Sex (F:M)	21:20	21:19	22:19
Assessment age —Mean (yr)	54.4	23.2	41.8
—Range	28–79	9–53	18–66
Onset age —Mean (yr)	42.0	4.2	9.9
—Range	1–69	0–16	0–45
Allergic provoking factors	None	All	22
Allergic rhinitis/eczema	None	31	20
No positive skin tests—Mean (Total 23)	0	9.5	7.9
—Range	0	5–21	3–17
Radiographic abnormality—			
Transient shadows			13
+ Fixed shadows	None	None	18
+ Bronchiectasis			10
Serum total IgE (IU/ml) —Mean	212	1348	4058
—Range	8–1750	56–4700	52–23500

ASPERGILLUS PRECIPITINS

The presence of serum precipitins to *Aspergillus* and *Candida* species were detected by a double diffusion technique (Longbottom and Pepys, 1964).

SERUM TOTAL IGE

Total IgE was measured by a Sepharose particle radioimmunosorbent test (SPRIST) (Cromwell *et al.*, 1979).

HLA TYPING

Ten ml of heparinised blood was taken. Lymphocytes were separated over Ficoll-Triosil and stored in liquid nitrogen until HLA typed. A standard two-stage microlymphocytotoxicity method was used (Kissmeyer-Nielsen and Kierbye, 1967). To each 2000 lymphocytes 1 μ l of antiserum and 5 μ l of complement were added. Dead and living cells were scored by Trypan blue exclusion; 115 sera were used, obtained from screening of local pregnant women and the NIH bank. The sera conformed to the international standards of the 7th Histocompatibility Workshop, Oxford, 1977. These sera defined 14 antigens of the A locus, 17 antigens of the B locus, BW4, and BW6, and 5 loci of the C locus. All patients were ABO and rhesus-blood grouped.

ANALYSIS OF HLA POPULATION STUDIES

The frequency of the HLA antigens in each group was compared with the frequencies in other groups and with 167 healthy hospital and laboratory workers using the Chi-square test with Yates correction.

Probability values of 0.05–0.001 should be considered critically because of the large number of comparisons made unless a previous study has suggested a particular antigen to be of a priori

Table 2 HLA haplotypes in asthma families

Family no	Paternal haplotypes						Maternal haplotypes						Haplotype propositus	Other associated disease haplotype	Affected			Unaffected				
	"a"			"b"			"c"			"d"					2	1	0	2	1	0		
	A	B	C	A	B	C	A	B	C	A	B	C										
1		2	7	W19	13		28	5		3	40	W2	a/c	b/d			1		1			
2		2	27	W2	W29	W44		1	8		2	W44	a/c	b/d			2		2			
3		2	W35	W4		2	W44	W4		1	7		a/c	b		1		2	2			
4	II	26	W38		3	5		W19	27	W2	W19	27	a/c	—		1						
	III	1	W35		1	17		W19	27		26	W38	b/d	—					2			
5		1	5		2	13		26	W35		W26	W22	a/c	b/d		1	1		1			
6		2	W44	W5	2	7		1	8		2	7	a/c	d		1	1					
7	I	3	15	W3		?		3	8		W31	15	a/c	—					1			
	II	2	8		W24	7		3	8		3	15	W3	b'/c	d'		1					
8		2	W44		1	W44		W24	W35		1	37	a/c	d		1	2		1			
9	I	3	W35		11	40		1	40		1	40	b/c	—		1			2			
	II	3	W35		1	40		W24	W38		W32	40	W2	a/c	d			1	2			
10	I	1	8		11	7		W23	7		2	W44	a/c'	—		1			1			
	II	1	8		11	7		2	14		2	W39	a/c	b/d		2	1	1	1			
															Total		8	6	6	2	11	6

interest. P values may be "corrected" by multiplying by the number of comparisons made (Vladutiu and Rose, 1974). Relative risks were calculated to give an index of frequency of disease in those subjects carrying an antigen in relation to those not.

ANALYSIS OF HLA FAMILY STUDIES

The principle of the analysis is the segregation of HLA haplotypes in asthmatic, atopic, and unaffected relatives.

The figure shows the family pedigrees. The haplotypes for each family were deduced by inspecting the pedigree to see which antigens at different loci occurred together consistently. Paternal haplotypes are represented by letters "a" and "b" and maternal haplotypes by "c" and "d." These letters were assigned so that the haplotypes of the propositus were "a/c." The antigens constituting each haplotype in the families can be seen in table 2, columns two to five. Different generations within a family must be considered separately because a child must inherit one or other haplotype from a parent, and this gives the measures a degree of dependence. The "secondary" generation parents are represented by "a'/b'" and "c'/d.'" Thus a single haplotype may be represented by two codes, for example b'/c', so that the generations can be considered separately. The propositus of each family is indicated by an arrow. The index case for the secondary generations is the parent or child of the propositus unless that individual is unaffected. The eldest appropriate member of the secondary generation is then taken as the index case.

For the asthmatic propositi, the number of asthmatic and non-asthmatic siblings sharing both, one, or neither haplotype are tabulated separately in table 2. The overall distribution for the degree of haplotype sharing, or segregation, for all the families is found by summing those sharing both, one, and neither haplotype. The predicted segregation is calculated taking account of generations where not all of the haplotypes could be ascertained. The observed and predicted distributions were compared using the distribution Chi-square test (table 3).

Similar analyses were done taking atopic propositi and considering atopic and non-atopic relatives, and taking propositi with raised serum total IgE concentrations and considering relatives with normal and raised IgE.

Results

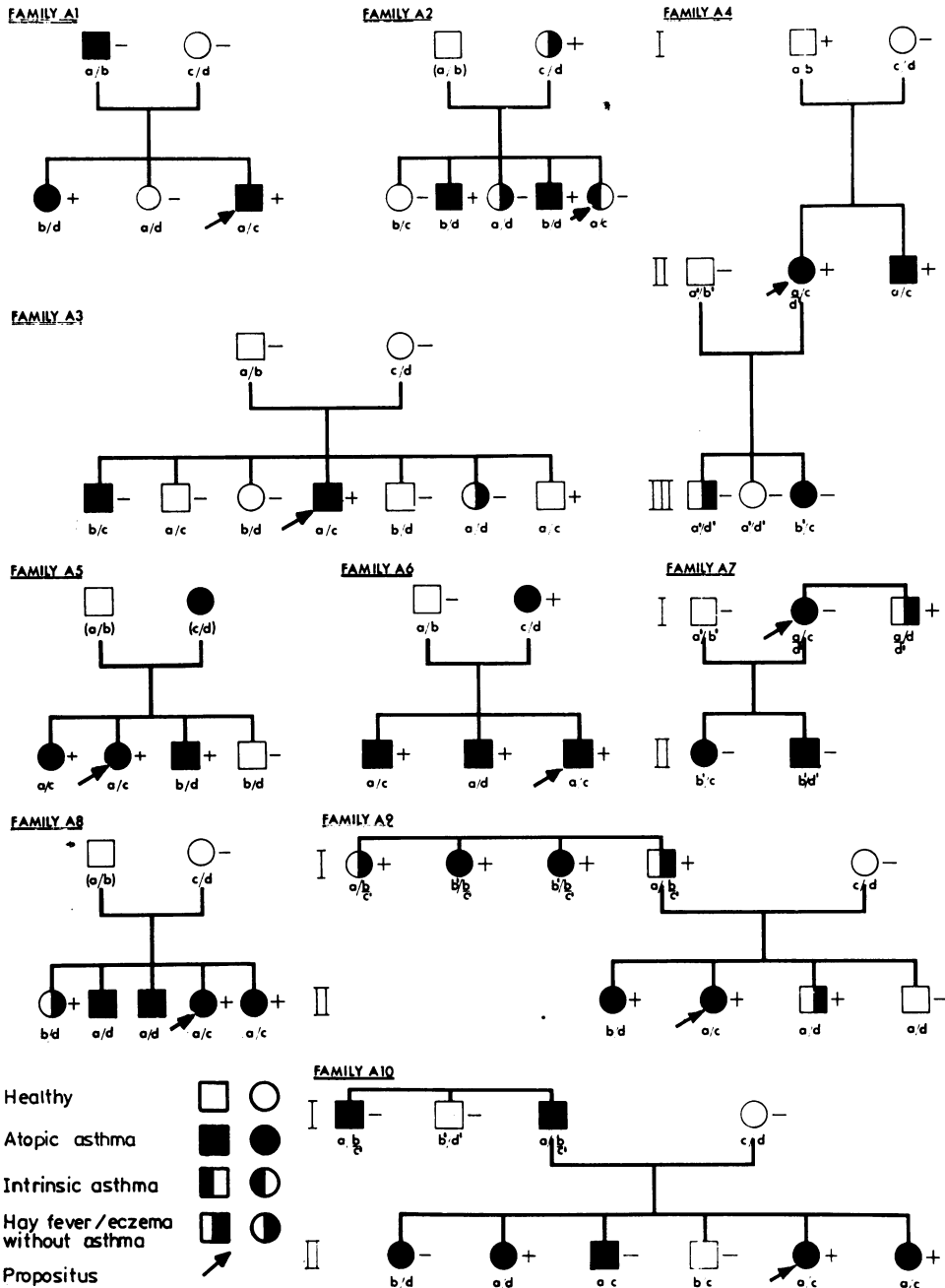
HLA POPULATION STUDIES

The HLA frequencies in general did not differ significantly when the whole asthma group or the asthma subgroups were compared with controls or other subgroups. The exceptions are shown in table 4. The frequencies, probability values, and relative risks are shown for those comparisons that were significant before correction. None of the probabilities is significant after correction for the number of comparisons made. Full details of all HLA frequencies for the various groups are available on request.

HLA FAMILY STUDIES

In table 3 the families are considered together and compared with the segregation predicted for a

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HLA haplotypes are represented by small letters and antigens are identified fully in table 2 (see text). + indicates raised serum total IgE and - normal total IgE. Parentheses () indicate that subject was not available and haplotypes have where possible been deduced from segregation in rest of family, or assumed.

Table 3 *Haplotype sharing in asthmatic families*

Propositus	Group		Total	Haplotypes shared			Distribution χ^2	P	
				2	1	0		>0.1	<0.5
Asthmatic	Asthmatic siblings	Observed	20	8	6	6	3.91	NS	<0.5
		Predicted		5	10.25	4.75			
Asthmatic	Non-asthmatic siblings	Observed	19	2	11	6	2.88	NS	<0.5
		Predicted		4.75	10	4.25			
Atopic	Atopic siblings	Observed	27	9	12	6	1.11	NS	<0.9
		Predicted		6.75	14.25	6			
Atopic	Non-atopic siblings	Observed	12	3	4	5	2.0	NS	<0.5
		Predicted		3	6	3			
↑ IgE	↑ IgE siblings	Observed	17	7	7	3	2.40	NS	<0.5
		Predicted		4.25	9.25	3.5			
↑ IgE	Normal IgE siblings	Observed	15	3	8	4	0.22	NS	<0.9
		Predicted		3.75	7.75	3.5			

null hypothesis of no haplotype association, having regard for generations where not all of the haplotypes have been ascertained. The segregation does not differ significantly from that predicted. Five of the ten families are not consistent with a single postulated asthma associated haplotype, and in families of this size the consistency in the other families could be due to chance.

The analysis was repeated in a similar way for the atopic and non-atopic siblings of atopic index cases, and siblings with normal and raised total IgE concentrations of index cases with raised total IgE concentrations. There are no significant differences between the observed and predicted segregations (table 3).

Table 4 *Differences in HLA frequencies within asthma subgroups and between controls*

Groups compared	HLA	Frequencies %	Frequencies %	P	Relative risk
Intrinsic/control	B8	41	23	<0.05	2.7
Extrinsic/control	B15	25	8	<0.02	3.6
ABPA/control	B7	15	32	<0.05	0.36
Normal IgE/high IgE	A2	77	49	<0.01	3.5

P values are uncorrected for the number of comparisons made.

Discussion

Care has been taken to define populations of asthmatics that are clinically either definitely extrinsic atopic or intrinsic. ABPA is of particular immunological interest, offering an opportunity to investigate response to a well-defined antigen, and is of clinical importance as a cause of irreversible airways obstruction. The results of the population studies reported here suggest that there is no real association between asthma and the HLA A, B, or C loci. There is no convincing a priori interest in published reports for the antigens

where differences were found, and none of the differences were significant after correction for the number of comparisons made. The work of Thorsby *et al* (1971) has been cited as showing an increased frequency of A1/B8 in asthmatic children, but the figures were not significant at the 5% level even before consideration of the number of comparisons made. The lack of association with serum total IgE concentrations is consistent with published reports.

Brostoff *et al* (1976) reported an increased frequency of BW6 in intrinsic asthma and suggested that this was evidence for a recessive mode of inheritance. Their study can be criticised in that the numbers were small and included patients with chronic bronchitis. No correction for the number of comparisons was made. The justification of conclusions about patterns of inheritance without family studies is doubtful. Our results show no evidence for this association even when only those patients resistant to treatment with sodium cromoglycate are considered.

Turner *et al* (1977) reported differing frequencies of common HLA antigen combinations in some atopic syndromes. A1 with B8 was increased in eczema associated with hay fever or asthma, but not in eczema, hay fever, or asthma alone. A1/B8 was considered of a priori interest after Thorsby *et al* (1971), so no correction for the number of comparisons was made. Our results show a trend for this combination to occur more often in the presence of eczema, but this was not significant.

Family studies can help in defining weak HLA disease associations and have other advantages over population studies. Genetic and environmental differences within families are more limited than in the general population. HLA disease associations should be found if they exist as it would be expected that genetic influence in multi-

factorial disease would be particularly important in families where several siblings are affected. When associations are found conclusions can be made about the type to gene inheritance, penetrance, and linkage.

Family studies also have their problems. Suitable families are unusual, and important members may be inaccessible so numbers tend to be small. All the haplotypes may not be identified if only one generation is available. Allowance then must be made when the predicted segregation is calculated, or it is assumed that all four haplotypes are different. Illegitimacy complicates analysis and supporting evidence of paternity from consistent ABO and rhesus blood group inheritance is important. There is a degree of dependence of the measures, and care must be taken in combining analysis of different generations. Assumptions are made when considering unaffected relatives about disease that has not been expressed clinically or is expressed subsequently. Haplotype segregation in unaffected members will be altered only if there is an association and the gene penetrance is high.

The reports of family studies suggesting linkage between HLA haplotypes and hypersensitivity to ragweed antigens created wide interest (Levine *et al*, 1972; Blumenthal *et al*, 1974). Black *et al* (1976) was unable to confirm these findings in a study of a larger number of small families. Wagatsuma *et al* (1976) reported an association between HLA haplotypes and asthma but the way in which different generations were analysed together can be criticised.

The family studies reported here show no significant HLA haplotype association with asthma or atopy. Five of the ten families are not consistent with a postulated asthma associated haplotype. The observation that the asthma affected siblings of asthmatic propositi share both haplotypes more often than asthma unaffected siblings does not exclude the possibility of association in some families, possibly with highly specific immune response genes.

It is concluded that no associations of overall biological importance between HLA and asthma have been found. Genetic influences in asthma are very complex and study of other markers, genetic, immunological, and biochemical will be needed to elucidate the mechanisms of these influences.

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