

HLA-A, B and C and HLA-DR antigens in intrinsic and allergic asthma

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Summary

Some 103 patients with asthma and 100 healthy volunteers have been typed for HLA-A, B and C and HLA-DR antigens. The 103 patients consisted of thirty-three with intrinsic asthma, thirty-four with extrinsic asthma, and thirty-six known to have precipitins to *Aspergillus fumigatus*. No increase in frequency of any of the A, B, C, or DR antigens was found to be significant after correction for the number of comparisons was made. However certain trends comparable to findings in other immunopathic disorders were noted. For example B12 was increased in the allergic asthmatics (46 vs 29% controls) and it is suggested that B12 is associated with the ability to produce the IgE antibodies. A3/B7/DRw2 (which are in linkage disequilibrium) all show a decreased frequency in intrinsic asthma (24, 12 and 9% vs 32, 26 and 24% respectively in controls). Finally B8 and DRw3, which showed a moderate increase in frequency in all three groups of asthmatics, were found in five of seven patients with low atopy but persisting antibodies to *A. fumigatus*. Further detailed studies of these asthmatic subgroups is warranted.

Introduction

It is becoming increasingly understood that more than one genetic locus is involved in determining familial predisposition to asthma. One such locus may be within the HLA system since a number of studies have suggested an association between HLA antigens and asthma. Thorsby, Engeset & Lie (1971) found an increase (which was not statistically significant) of the haplotype A1-B8 in childhood asthmatics, and they suggested that this haplotype was associated with a subgroup of asthmatics, namely the non-allergic intrinsic group. In a previous report of 100 asthmatics Morris M.J. *et al.* (1977) showed a non-significant increase of A1 and B8 in extrinsic asthmatics, and a decrease of B12 in intrinsic asthmatics. Furthermore, a small subgroup of patients with negative skin tests but a specific history of allergy precipitating asthma had the highest fre-

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quency of A1-B8. Rachelefsky *et al.* (1976) studied the frequency of B-cell antigens in childhood asthmatics and found one (designated Group 2) to be significantly increased in the patients compared to the control panel. Subsequently, this B-cell antigen was shown by the same authors not to be an HLA-DR antigen, and in fact does not represent a distinct antigen at all. Brostoff *et al.* (1976) found in a small number of intrinsic asthmatics that 81% were homozygous for the 'public' antigen HLA BW6 (the expected frequency was 36%).

Thus the results of studies reported so far do suggest that there may be some association between HLA and predisposition to asthma, although this association is certainly not a strong one.

We have studied a further 103 adult asthma patients with two objectives in mind. First to examine the subgroup of patients with low atopy (negative skin tests and low IgE levels), but with a documented allergic mechanism, i.e. type III hypersensitivity to *A. fumigatus*. Secondly, to see if there was a closer association between asthma and the more recently defined HLA-DR antigens than with the HLA-A and B antigens.

Materials and methods

Patients

All patients were Caucasian and were selected from an adult outpatient asthma clinic. The purpose of the study was explained to each patient and permission obtained for inclusion in the study. Asthma was defined as variable wheezing and/or dyspnoea, which reversed spontaneously or after treatment, at some time. The asthma tended to be moderately severe and chronic in all patients. Three polar groups of asthmatics were selected, based on the following criteria:

Group I. (Intrinsic). Thirty-three asthmatics were selected in whom no skin tests were positive and no history of allergy nor of any associated atopic condition was elicited. Mean total IgE concentration was 37 ± 34 ($\pm$ s.d.) i.u.; mean peripheral blood eosinophil count in twenty-eight patients was $325 \pm 370 \times 10^6/\text{litre}$.

Group II (Extrinsic atopic). Thirty-four asthmatics were selected in whom there were three or more positive skin tests with weals 2 mm or greater in diameter. The average number of positive skin tests in this group of patients was 5.25. These patients did not have precipitins to *A. fumigatus*. The mean total IgE concentration was greater than 346 ± 313 (see IgE determination), and the mean eosinophil count in thirty patients was $378 \pm 295 \times 10^6/\text{litre}$.

Group III (Aspergillus precipitins). Thirty six asthmatics known to have had precipitins to *A. fumigatus* were selected. The average number of positive skin tests was 3.1. The mean total IgE was greater than 330.4 ± 271.3 i.u., and the mean eosinophil count in twenty patients was $567 \pm 664 \times 10^6/\text{litre}$.

The following subgroups were selected out of the Group III asthmatics.

(i) Allergic broncho-pulmonary aspergillosis (ABPA). The criteria for this diagnosis were (a) precipitins to *A. fumigatus* (b) positive skin test to *A. fumigatus*, (c) high total IgE level (d) blood eosinophilia, and (e) persistent fleeting shadows and/or in some cases progressive chest radiograph changes of collapse, fibrosis, proximal bronchiectasis, or loss of lung volume. The diagnosis of ABPA was made in twenty-one patients, sixteen of whom had evidence of fleeting shadows with or without radiographic evidence of persistent lung damage. In the remaining five there was no documentation of fleeting shadows, but the other X-ray changes were present. In these

twenty-one patients mean IgE level was greater than 448 ± 229 i.u. and the mean eosinophil count in fourteen patients was $895 \pm 747 \times 10^6/\text{litre}$.

(ii) Patients with persistent precipitins to *A. fumigatus*. Only thirty of these group III patients had precipitins to *A. fumigatus* at the time of this study even though they had all had them at an earlier date.

(iii) Patients with low IgE (< 125 i.u.). ($n = 10$) Only 10 patients in group III had low IgE levels, mean 58 ± 37 i.u. In these 10 patients the average number of skin tests positive was 1.6 with a mean weal size of 1.9 mm, compared with the rest of group III ($n = 26$) in whom the mean IgE was greater than 434 ± 248 i.u., and the mean number of skin tests positive was four with a mean weal size of 2.4 mm. Seven patients in group III had low IgE and persistent precipitins to *A. fumigatus*. In this group of seven, mean IgE level was 64 ± 40 i.u., mean number of prick tests positive was 1.3 with a mean weal size of 1.7 mm.

Control population

One hundred unrelated healthy Oxford Caucasians made up the control group. All were HLA typed during the time of the asthmatic study with the same antisera.

IgE determinations

Serum IgE levels were measured by the Phadebas IgE PRIST kit method (Pharmacia Diagnostic, UB Uppsala, Sweden). A level of 125 i.u./ml was taken as the upper limit of normal. The IgE figures quoted for groups II and III underestimate the actual means because IgE levels above 1000 i.u./ml were not measured precisely but were taken as being 1000 i.u./ml in the calculation of mean values.

Precipitin tests

These were done using agar gel double diffusion (Faux *et al.*, 1974) and four extracts of *A. fumigatus*. Two were supplied by Bencard, Brentford, Middlesex, one by Dr W. Brighton at the National Institute for Biological Standards, and another was prepared by J.A.F. from a strain of *A. fumigatus* isolated from a patient at the Churchill Hospital, Oxford. All 103 sera were tested.

Negative sera in group II and group III were concentrated three-fold and then retested.

HLA typing

(i) *HLA-A and B antigens*. The following thirty-six antigens were tested for in all of the subjects:

A locus—A1, 2, 3, 11, 25, 26, 28, 29; Aw23, 24, 30, 31, 32, 33.

B locus—B5, 7, 8, 12, 13, 14, 15, 17, 18, 27, 37, 40; Bw21, 22, 35, 38, 39.

C locus—CW1, 2, 3, 4, 5.

(ii) *HLA-DR typing*. All of the subjects were typed for DRw1, 2, 3, 4, 6, and 7. DRw5 was tested for in all of the control panel and in about half of the asthma patients. Over seventy sera were used to define the antigens. At least two 'operationally monospecific' sera were used to define each specificity with the exception of DRw6; all DRw6 antisera contained in addition, either anti DRw1 and 2, anti DRw2, or anti DRw5. The sera were characterized as having a given specificity or specificities by screening each serum against a panel of random volunteers who were typed with highly

selected sera from the U.K. Regional Workshop (Bodmer, Pickbourne & Bodmer, 1976) and the seventh International Workshop (Bodmer *et al.*, 1978).

(iii) *Cytotoxicity test.* The standard NIH eosin dye exclusion test was used for the detection of the HLA-A, B and C antigens. The HLA-DR antigens were detected with the same cytotoxic test but with a 2 hr incubation step after the addition of rabbit complement.

(iv) *Lymphocyte preparation.* Lymphocytes were isolated from defibrinated blood by the conventional density gradient centrifugation method (Harris & Okaejiofo, 1969). B lymphocytes were separated from T lymphocytes by rosetting the T lymphocytes with papain-treated sheep red blood cells (Wilson, Haegert & Coombs, 1975), followed by density gradient centrifugation. The purity of the B lymphocyte preparation was monitored in the cytotoxic test by a rabbit anti-human B lymphocyte serum (kindly provided by Professor W. F. Bodmer) and a multispecific anti-DR pregnancy serum.

(v) *Statistical analyses.* Differences in antigen frequency between any two groups were calculated by the Chi-squared test incorporating Yates correction factor.

Skin testing

Prick testing method was used with the allergens of the Bencard panel, including cat, dog, feathers, egg, milk, grasses, *A. fumigatus*, Oxford variant of *A. fumigatus*, cladosporium, house dust and house-dust mite. If suggested by the history, further allergens were tested. A 1 mm weal or greater was taken as positive, though in the highly atopic Group II all who were selected had weals of at least 2 mm in diameter.

Results

The antigen frequencies in the main groups of asthmatics and in the control subjects are shown in Table 1. All DRw antigen frequencies are shown, and those of the A, B and C loci that could be of interest in asthma. There are no significant differences (after a correction factor has been applied for the number of comparisons made) in the

Table 1.

Antigen	Controls (n=100)	Combined Asthma (n=103)	Frequency (%)			
			Group I Intrinsic (n=33)	Group II Extrinsic (n=34)	Group III Type III (n=36)	Group II+ Group III (n=70)
A1	26.0	29.1	24.2	29.4	33.3	31.4
A3	32.0	24.3	12.1	32.4	27.8	30.0
B7	26.0	23.2	12.1	26.5	30.6	28.5
B8	20.0	30.1	30.3	26.5	33.3	30.0
B12	29.0	40.8	30.3	50.0	41.7	45.7
DRw1	24.0	16.5	15.2	20.6	13.9	17.1
DRw2	24.0	19.4	9.1	20.6	27.8	18.6
DRw3	19.0	30.1	30.3	29.4	30.6	30.0
DRw4	36.0	44.7	42.4	47.1	44.4	45.7
DRw5	7.0	6.7	6.7	0	11.1	2.9
DRw6	15.0	15.5	9.1	14.7	22.2	18.6
DRw7	30.0	27.2	24.2	35.3	22.2	28.6

Table 2. Subgroups of Group III (asthma + precipitins)

Antigen	Frequency (%)					
	Controls (n=100)	ABPA (n=21)	Low IgE (n=10)	Persistent precipitins		
				High IgE n=30	Low IgE n=23	Low IgE n=7
A1	26.0	42.9	40.0	40.0	34.8	57.1
A3	32.0	23.8	20.0	30.0	34.8	14.3
B7	26.0	28.6	30.0	26.7	30.4	28.6
B8	20.0	28.6	50.0	36.7	26.1	71.4
B12	29.0	47.6	40.0	40.0	34.8	42.9
DRw1	24.0	14.3	0	13.3	17.4	0
DRw2	24.0	33.3	0	30.0	39.0	0
DRw3	19.0	23.8	50.0	33.3	21.7	71.4
DRw4	36.0	47.6	40.0	40.0	47.8	28.5
DRw5	7.0	7.1	10.0	6.7	4.3	14.3
DRw6	25.0	14.3	30.0	20.0	21.7	28.6
DRw7	30.0	19.0	30.0	20.0	17.3	14.3

frequencies of any antigen between the control group and all the asthmatics grouped together or any of the three main subgroups. However, there are certain trends in the data that are worthy of note. The frequency of B8 is higher in the total group of asthmatics as well as in groups I, II and III, compared to the control group and DRw3 shows a similar increase in frequency. The frequency of B12 is higher in groups II (extrinsic) and III (*Aspergillus* precipitins) than in the control group and group I (intrinsic). When all the allergic asthmatics, i.e. groups II and III, are taken together the increased frequency of B12 against the control group approaches statistical significance ($P < 0.05$ without correction for the number of comparisons). Eleven patients in group II (extrinsic), who had positive prick tests to *A. fumigatus* but to precipitins had a more marked increase in the incidence of B12 (64%). There is also a substantial decrease in the frequency of HLA-A3, B7, and DRw2 in group I (intrinsic).

The number of patients in some of the sub divisions of the *Aspergillus* precipitins group are too small for statistical analysis but again some trends emerge (Table 2).

(i) *ABPA*. In this group there is a non-significant increase in the frequency of antigens A1 and B12.

(ii) Persistent producers of precipitins to *A. fumigatus*. In this sub-group ($n=30$) there is again a trend for the frequency of antigens A1, B8, DRw3 and B12 to be increased.

(iii) Patients with low IgE. Of the ten asthmatics in Group III with low IgE, five were B8, DRw3 (50%), while all were DRw1 and DRw2 negative. Only seven of the ten were persistent producers of IgG precipitating antibodies to *A. fumigatus*; and of this seven, five were B8 DRw3 (71%).

Discussion

In this study of 103 asthmatic patients, there was no definite association with HLA. More specifically we failed to show a significant association with B8 in the total patient group, in the extrinsic asthma group, an association previously suggested by ourselves,

Morris *et al.* (1977) or in the intrinsic group as previously suggested by Thorsby *et al.* (1971). Nor was there any closer association with DRw3, an antigen which is in linkage disequilibrium with B8.

Of great interest was the increase in B12 in Group II (extrinsic atopic) and Group III (*A. fumigatus* precipitins) patients. An increased frequency of B12 was found by Thompson *et al.* (1976) in atopic children with steroid sensitive nephrotic syndrome. The increase in B12 may be associated with a general ability to respond to external allergens either by production of IgE and/or short term sensitizing IgG and precipitating IgG antibodies. The particularly high incidence of B12 (64%) in patients who were prick test positive to *A. fumigatus* but did not produce precipitating antibodies suggests that it is the ability to produce IgE antibodies that is associated with B12.

The decreased frequency of A3, B7 and DRw2 (antigens which are in linkage disequilibrium) in intrinsic asthma may also be an observation worthy of further study. B7 has been associated with allergy to ragweed (Marsh *et al.*, 1973), and a heightened immune response to flagellin (Morris P.J. *et al.*, 1977), while a decrease in DRw2 has been noted in rheumatoid patients with low or absent titres of rheumatoid factor (Panayi, Wooley & Batchelor, 1978). On the other hand chronic active hepatitis patients with B7 are deficient in autoantibodies to smooth muscle (Eddleston & Williams, 1978). Thus there is some evidence of associations of the B7/DRw2 haplotype with immune responsiveness or the lack of it, and so it is possible that some genetic influence associated with this haplotype is involved in the development of intrinsic asthma.

The attempt in this study to find a group of patients who were not atopic but in whom allergic mechanisms were involved in the genesis of their asthma was not particularly successful. However, in the small group of seven with low atopy but with persistent precipitating antibodies to *A. fumigatus*, five were B8, DRw3 and all were negative for DRw1 and DRw2. This is a small number of patients but an intriguing comparison can be made to findings in a number of other immunopathic disorders, where there is an association between persistence in titre of autoantibody and B8 (and presumably DRw3), e.g. diabetes and islet autoantibodies (Morris *et al.*, 1976), thyrotoxicosis and thyroid antibodies (Irvine *et al.*, 1977) rheumatoid arthritis and rheumatoid factor (Panayi, Wooley & Batchelor, 1978). There is always difficulty in choosing an acceptable cut-off value for IgE between high and low atopy. In our patients a natural gap occurred at 125 units. At the time of the study none of these patients were currently having, or had recently had, desensitizing injections. The IgE levels of most patients were measured in spring or summer.

In this study we have found no statistically significant association between asthma and HLA, but we have been able to demonstrate certain changes in antigen frequency which are of particular interest because of their similarity to findings in other immunopathic disorders. None of the HLA associations with disease has been shown to be absolute, and it is possible that in asthma the HLA association is only with a particular subset of the individuals with the disease. The genetic control of atopy may mask a genetic association of some other immunological mechanism in asthma.

The tantalizing speculation remains that in patients with allergic asthma, mechanisms other than those mediated by IgE may be associated with B8 and DRw3, and that B12 may be associated with the production of IgE. Yet a different genetic influence is seen in the intrinsic asthmatics and this may be associated with the absence of B7 and DRw2. Further detailed studies of the defined subgroups of asthmatic patients is warranted.

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