

Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes

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Summary

Background Aspirin-induced asthma (AIA) affects one in 10 individuals with adult-onset asthma. It is not known if aspirin sensitivity is due to immune mechanisms or to interference with biochemical pathways.

Objective The study aimed to test for possible involvement of the genes of the Major Histocompatibility Complex (MHC) in AIA.

Methods HLA-DPB1 and HLA-DRB1 genotyping was carried out by DNA methods in 59 patients with positive challenge tests for AIA and in 48 normal and 57 asthmatic controls.

Results The DPB1*0301 frequency was increased in AIA patients when compared with normal controls (19.5% vs 5.2%, Odds Ratio = 4.4, 95% Confidence Interval (CI) 1.6–12.1, $P=0.002$), and compared with asthmatic controls (4.4%, OR = 5.3, 95%CI = 1.9–14.4, $P=0.0001$). The frequency of DPB1*0401 in AIA subjects was decreased when compared with normal controls (28.8% vs 49.0%, OR = 0.42, 95%CI = 0.24–0.74, $P=0.003$) and asthmatic controls (45.6%, OR = 0.48, 95%CI = 0.28–0.83, $P=0.008$). The results remained significant when corrected for multiple comparisons. There were no significant HLA-DRB1 associations with AIA.

Conclusion The presence of an HLA association suggests that immune recognition of an unknown antigen may be part of the aetiology of AIA.

Keywords: aspirin-induced asthma, biochemical pathway

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Introduction

Aspirin-induced asthma (AIA) affects approximately 10% of adult asthmatics [1]. In susceptible individuals, ingestion of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) precipitates bronchoconstriction which may be violent or even fatal. The disease is more common in females than in males, and usually occurs after the third decade.

The aetiology of AIA is unknown. Many studies have focused on the inhibition of cyclo-oxygenase (COX) by NSAIDs. COX is involved in the metabolism of arachidonic acid to prostaglandins. Raised levels of the bronchoconstrictor Leukotriene E_4 (LTE₄), a metabolite of arachidonic

acid via the 5-lipoxygenase pathway, are detected in AIA patients after NSAID exposure [2–4]. Inhibition of 5-lipoxygenase prevents the production of leukotrienes and prevents or attenuates AIA in affected patients [5]. However, LTE₄ is also detected in the urine of NSAID-tolerant asthmatics after an asthmatic attack [6,7], and symptomatic asthma persists in AIA patients who are not ingesting NSAIDs.

The age of onset of AIA, and the usual absence of a family history, suggest that any metabolic abnormalities are acquired. AIA may therefore represent a progressive form of asthma which develops independently of NSAID exposure [8], but which is precipitated by the effects of NSAIDs in susceptible individuals.

Conversely, the reaction to aspirin and similar compounds in patients with AIA may be due to immune

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mechanisms rather than interference with biochemical pathways. AIA is not considered a result of atopic allergy, as Immunoglobulin E (IgE) antibodies to aspirin or other NSAIDs cannot be detected, and skin-prick tests are negative. Nevertheless, eosinophilia is a prominent feature of the disease, and airway inflammation could result from non-IgE mediated reactions to an endogenous or exogenous antigen.

The HLA Class II genes of the human MHC are central to the immune processing of exogenous antigens, and the finding of an HLA association with AIA might favour immunity as the underlying mechanism of the disease. Previous studies of HLA association in AIA have given conflicting findings which have not generally been confirmed [9–12]. We have therefore investigated a relatively large sample of patients with AIA from the Polish population, comparing them to normal healthy controls and aspirin tolerant asthmatic controls.

Materials and methods

Subjects

Three groups of subjects were investigated from a Polish population.

Group 1

Fifty-nine subjects with aspirin-induced asthma (44 women and 15 men, mean age 48.5 years, range 26–73 years). All subjects were asthmatic: there were no cases whose symptoms were restricted to nose or skin.

Group 2

A first control group of 48 healthy subjects, consisting of laboratory and hospital employees. The controls were approximately matched for age and sex with the AIA subjects (33 women and 15 men, mean age 40.6 years, range 23–58).

Group 3

A second control group of 57 aspirin-tolerant asthmatics (33 women and 24 men, mean age 45.6 years, range 29–71).

Methods

Aspirin intolerance was confirmed in all AIA subjects by an oral, placebo-controlled challenge test with increasing doses of aspirin (10–320 mg), as previously described [13,14], or by a bronchial challenge test with increasing doses of aspirin (Aspisol, Bayer AG, Germany), as previously described [15]. When necessary, challenge tests were also performed in control asthmatics, to exclude aspirin

hypersensitivity. All patients were asked about a family history of AIA, and in all cases the answer was negative.

Skin-prick tests with 16 common allergens were performed in all subjects (SmithKline Beecham, UK). The total IgE was determined by nephelometry (Behring, Germany), and specific IgE to five aero-allergens was carried out by the FAST method.

All asthmatics participating in the study were non-smokers in a stable clinical condition, who had been followed as outpatients for at least 6 months. All subjects gave informed consent to the studies, and the protocols were approved by the local ethics committees.

DNA was extracted from washed buffy coat cell pellets using a Genepure Nucleic Acid Purification System – Model 341 (Applied Biosystems Inc., Warrington, UK). HLA-DPB1 typing of 19 recognized alleles [16] was performed by restriction enzyme analysis of the second exon of DPB1 amplified by PCR as described [17]. HLA-DRB1 typing of 12 alleles was performed using sequence-specific oligonucleotide probing of PCR products as described previously [18]. The frequency of 12 DRB1 and 19 DPB1 alleles were calculated for the aspirin-sensitive, normal control and asthmatic control groups of both populations.

Odds ratios for comparisons of allele frequencies, 95% Confidence Intervals, and two-tailed *P*-values were calculated by the STATXACT program (Cytel Corporation, Cambridge MA, USA). Rare alleles, or alleles absent from the subjects were not tested for association. *P*-values were corrected for multiple comparisons by multiplying by 10, as 10 alleles at the HLA-DP and DR loci were sufficiently common to test for association with AIA.

Results

HLA-DP alleles

The frequency of HLA-DPB1*0301 was 19.5% in the AIA group, compared with 5.2% in the normal controls (OR=4.4, 95%CI=1.6–12.1, *P*=0.002), and 4.4% in the asthmatic controls (OR=5.3, 95%CI=1.9–14.4, *P*=0.0004)(Table 1). These results were significant after *P*-values were corrected for multiple comparisons (*p*_c=0.02 and 0.004, respectively). There were no differences in the DPB1 allele frequencies between the normal and asthmatic controls. The frequency of the DPB1*0401 allele was 28.8% in the AIA group compared with 49.0% in normal controls (OR=0.42, 95%CI=0.24–0.74, *P*=0.003) and 45.6% in the asthmatic controls (OR=0.48, 95%CI=0.28–0.83, *P*=0.008). After multiple comparisons were taken into account, this negative DPB1 association compared with normal controls remained significant (*p*_c=0.03), although the comparison with asthmatic controls was no longer significant (*p*_c=0.08).

Table 1. Allele frequencies of DPB1 alleles

DPB1 Allele	AIA	Healthy controls	Asthmatic controls
*0101	12 [‡] (10.2%)	4 (4.2)	9 (7.9)
*0201	18 (15.3)	9 (9.4)	19 (16.7)
*0202	0 (0.0)	0 (0.0)	0 (0.0)
*0301	23 (19.5)	5 (5.2)	5 (4.4)
*0401	34 (28.8)	47 (49.0)	52 (45.6)
*0402	14 (11.9)	12 (12.5)	19 (16.7)
*0501	1 (0.8)	0 (0.0)	0 (0.0)
*0601	0 (0.0)	2 (2.1)	3 (2.6)
Other [§]	16 (13.6)	17 (17.7)	7 (6.1)
Total	118	96	114

[‡] Number of chromosomes (%)[§] Others = DBP1*0801, *0901, *1001, *1101, *1301, *1401, *1501, *1601, *1701, *1801 and *1901

HLA-DRB1 alleles

HLA-DRB1 amplification failed in nine subjects. In the remaining samples, the frequency of HLA-DRB1*11 was 10.9% in the AIA samples, compared with 21.3% in the normal controls (OR=0.45, $P=0.04$) and 20.8% in the asthmatic controls (OR=0.4, $P=0.05$) (Table 2). These changes were not significant when multiple testing was taken into account. Although the allele frequency of DRB1*03 in the AIA subjects was 12.7% compared with 5.3% in normal controls, the association was not significant.

No significant associations were found between the DPB1

and DRB1 loci and any skin test or specific IgE response, or the total serum IgE. These results indicated that the HLA-DPB1 association with AIA was independent of the effects of atopy.

Discussion

This study showed a strong positive association between the presence of HLA-DPB1*0301 and AIA, which has not been previously reported.

Krishnamoorthy *et al.* [11] showed a trend for an increase in DPB1*0301 frequency for AIA affected groups compared with healthy controls, but the sample was relatively small. The results of Lympany *et al.* [12] showed a possible increase in the frequency of DPB1*1401 in British AIA sufferers, but the sample size was again relatively small. Nevertheless, DPB1*1401 differs from DPB1*0301 by only a single amino acid in the first hypervariable region of the cell distal (β 1) domain, and it is possible that the two alleles may be functionally similar.

A negative association with DPB1*0401 was also significant in our study, in keeping with a previous report in British subjects [12]. Our results therefore confirm the hypothesis that DPB1*0401 is an independent protective factor for AIA.

Schmitz-Schumann *et al.* have previously described an increase in DRB1*03 alleles in Swiss-German subjects with AIA [15]. In the present study the trend was for DRB1*03 to be increased (14.2% in AIA subjects, and 4.9% in healthy controls), although the results were not significant. The findings are therefore consistent with the previous report [15], but confirmation of this association is required in larger independent samples.

We have not tested for the HLA-DQ loci in this study. However, HLA-DR is in strong linkage disequilibrium with HLA-DQ, whereas HLA-DP is only in weak disequilibrium, so that it is unlikely that a strong HLA-DQ association exists in these subjects. Nevertheless, direct testing of HLA-DQ will be necessary to exclude DQ involvement. In addition, the MHC contains many genes of unknown function, and it is possible that non-class II HLA genes within the MHC may account for the association seen in this study.

The HLA-DPB1*0301 association in our subjects is stronger than the HLA-DR relationships generally seen with allergens [19]. The relative risk of five for HLA-DPB1*0301 suggests that HLA typing might be clinically useful in the early recognition of potential aspirin sensitivity in adult onset asthmatics.

HLA associations have been seen in industrial lung disease due to other small molecules. HLA-DPB1*0201 is positively associated with Beryllium Disease [20],

Table 2. Allele frequencies of HLA-DRB1

DPB1 Allele	AIA	Healthy controls	Asthmatic controls
*01	7 [‡] (6.4%)	10 (10.6)	10 (9.4)
*02	15 (13.6)	9 (9.6)	13 (12.3)
*03	14 (12.7)	5 (5.3)	9 (8.5)
*04	16 (14.5)	13 (13.8)	20 (18.9)
*07	19 (17.3)	14 (14.9)	16 (15.1)
*08	6 (5.5)	5 (5.3)	6 (5.7)
*11	12 (10.9)	20 (21.3)	22 (20.8)
*13	11 (10.0)	11 (11.7)	4 (3.8)
*14	7 (6.4)	2 (2.1)	2 (1.9)
Other [§]	3 (2.7)	5 (5.3)	4 (3.8)
Total	110	94	106

[‡] Number of chromosomes (%).[§] Other = DRB1*09, *10, *12 and DRBR.

DQB1*0503 may be increased in subjects with isocyanate-induced asthma [21], and HLA-DRB1*03 is increased in workers with acid anhydride allergy and asthma [22]. In addition to Berylliosis, HLA-DP associations have been reported in subjects with chronic juvenile arthritis [23]. HLA-DP associations are however, not seen in IgE-mediated responses to common major allergens [19]. The relative role of HLA-DP in antigen presentation, compared to HLA-DR and HLA-DQ is unknown.

The strength of the association suggests HLA-DPB1*0301 may itself confer susceptibility to AIA. AIA may therefore be in part due to an inflammatory reaction to a specific antigen. The antigen could be aspirin itself. Although AIA can be caused by a variety of non-steroidal anti-inflammatory agents with a variety of chemical structures, these molecules all possess a conformation that affects the enzymes of the COX pathway, and are likely to share antigenic determinants at the sites that interact with the enzymes. Other possible antigens include an autoantigen or a chronic viral infection [24,25]. These last possibilities are supported by the finding of elevated markers of autoimmunity in patients with AIA [26].

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