

# Human Leukocyte Antigens in Occupational Allergy: A Possible Protective Effect of HLA-B16 in Laboratory Animal Allergy

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*Human leukocyte antigens (HLA-A, -B, -C, and -DR loci) and possible associations with occupational allergy to laboratory animals and atopy indicators were studied in laboratory animal workers with airway symptoms (n = 92) and in those who were symptom free (n = 27), as well as in a population reference group of blood donors in good health (n = 123). The laboratory animal workers, but not the population reference group, were allergologically examined with skin prick testing to common environmental and animal allergens together with measurement of total serum IgE levels. Seven HLA antigens, i.e., HLA-A9, -B5, -B12, -B16, -DR4, -DR5, and -Drw6, suggested possible associations with symptoms and/or atopy indicators. When correcting the p-values for the number of studied antigens, only the HLA-B16 differences remained statistically significant. HLA-B16 was elevated in symptom-free subjects compared to the population reference group and in subjects with serum IgE <10 kU/L. Subjects with serum IgE >100 kU/L and sensitized against environmental and/or laboratory animals, including LAA asthmatics, lacked HLA-B16. It is suggested that HLA-B16 or an immunosuppressive gene linked to HLA-B16 reduce the risk of producing IgE antibodies against animal protein allergens. However, our a priori hypothesis of a possible risk associated with HLA B15-DR4 could not be confirmed. © 1996 Wiley-Liss, Inc.*

**KEY WORDS:** atopic allergy, HLA-B16, HLA-linked immunosuppression, laboratory animal work, occupational asthma, serum IgE

## INTRODUCTION

Laboratory animal allergy (LAA) is generally considered an IgE-mediated disease. The prevalence of LAA in laboratory animal workers range from 11% to 44% [Sjöstedt et al., 1995]. The most common symptom is rhinoconjunctivitis, but contact urticaria is also a common symptom. Furthermore, according to our experience, about one-third of those with rhinoconjunctivitis will later develop asthma,

in many cases implying incapacitating symptoms, resulting in job leaving. We have earlier found that subjects with atopy indicators such as parental and childhood allergy, raised total serum IgE levels, as well as positive skin prick tests against environmental allergens, have an increased the risk of developing LAA, especially asthma [Sjöstedt and Willers, 1989; Sjöstedt et al., 1993].

Atopic individuals are more susceptible to asthma [Slovak and Hill, 1981; Venables et al., 1988]. For example, family studies have shown that atopy is under genetic control, but the hereditary mechanism is unclear [Hopkin, 1993]. Thus, hereditary factors seem to be of importance as predisposing factors for the development of LAA. Sibbald [1980] found significantly higher prevalence rates of atopic diseases in first-degree relatives of allergic (extrinsic) asthma patients than in nonallergic (intrinsic) asthma patients. Additional family studies revealed higher prevalence of atopic asthma in relatives of atopics compared to

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nonatopics. These findings propose an independent hereditary mechanism for asthma and atopy [Sibbald, 1991].

More than one chromosome might determine atopy [Huang and Marsh, 1993]. Cookson and co-workers found support for an autosomal inheritance linked to chromosome 11q [Cookson and Hopkin, 1988; Cookson et al., 1989]. The specific IgE response has been postulated to be linked to the human lymphocyte antigen (HLA) complex on chromosome 6 [Blumenthal et al., 1986]. On the contrary, regulation of total serum IgE is probably not linked to HLA [Meyers et al., 1983].

Several studies indicate that there are HLA-linked immunogenetic factors in respiratory allergy [Mehra et al., 1992]. For example, Marsh et al. [1982] have proposed an association between HLA-Dw2 and ragweed pollen allergy. Ansari et al. [1991] demonstrated a relationship between well-defined allergens and HLA-D genotypes. Recently, Young et al. [1995] have suggested an association between HLA-DR3 and specific IgE to inhaled acid anhydrides.

We have previously found that LAA asthmatics have an increased frequency of family history of allergy [Sjöstedt and Willers, 1989]. We have also suggested a possible association between HLA B15-DR4 and LAA [Löw et al., 1988]. In that preliminary analysis of the first 27 cases, we reported high frequencies for HLA-B15 and -DR4, especially in the LAA asthma subjects. A possible association between various atopy indicators and specific HLAs were not studied, however. Furthermore, we have clinically observed that separate laboratory workers, in spite of many years of heavy animal exposure, do not develop LAA. The aim of the present analysis of the complete material is to study possible positive or negative associations between different HLAs, LAA symptoms, and atopy criteria.

## MATERIAL AND METHODS

### Subjects

The individuals were employed at 29 different research laboratories in which animals were kept. During a period of 10 years, the total number of animal-exposed workers in these research laboratories, at Lund University in Lund and Malmö, was about 300. The exact number of exposed subjects is impossible to establish, because of shortcomings in personnel registration routines. All 300 subjects were screened with regard to airway symptoms and 92 cases with airway symptoms were identified. They had work-related symptoms caused by laboratory animals and/or airway irritants such as solvents and formaldehyde. Additionally, 27 symptom-free subjects of the animal exposed group were examined as referents. Of the total 119 subjects in contact with animals, 97 were laboratory technicians, 4 animal handlers, and 18 scientists.

The distribution of airway symptoms, i.e., rhinitis

**TABLE I.** Distribution of Airway Symptoms and Skin Prick Test Results Against Laboratory Animals in the Exposed Group (n = 119)

| Group                      | n  | Gender<br>(female/male) | Age<br>(mean) | Age<br>(range) | Current<br>smoking<br>(%) |
|----------------------------|----|-------------------------|---------------|----------------|---------------------------|
| Symptom free               | 27 | 26/1                    | 42            | 29–56          | 33                        |
| Airway symptoms            |    |                         |               |                |                           |
| Test positive <sup>a</sup> | 53 | 39/14                   | 38            | 22–62          | 19                        |
| LAA asthma                 | 28 | 20/8                    | 40            | 26–62          | 11                        |
| Test negative              | 39 | 33/6                    | 41            | 22–63          | 13                        |

<sup>a</sup>Skin prick test against laboratory animals  $\geq +1$ .

and/or asthma, skin prick test results against laboratory animals, sex, age, and current smoking in the studied population, is presented in Table I. In the airway symptom group with positive animal skin prick tests, one subgroup had LAA asthma (n = 28) and the other LAA rhinitis (n = 25), respectively. In the test negative group, 17 subjects had asthma and 22 rhinitis symptoms.

A population reference group were examined according to the routines of the Blood Centre at Lund University Hospital. The reference group consisted of 123 subjects in good health. They were blood donors, both unrelated and unselected, concerning atopy.

### Allergy Workup

The cases (n = 92) and the symptom-free group (n = 27) were subjected to allergological investigation with questionnaire, skin prick testing against laboratory animals (7 species, i.e., rat, mouse, guinea pig, hamster, cat, rabbit, and pig), common environmental allergens (13 antigens), total serum IgE levels, and lung function tests as described earlier [Sjöstedt and Willers, 1989]. The population reference group has not been investigated allergologically.

### HLA Testing

HLA typing for HLA-A, -B, -C, and -DR loci has been performed with lymphocytotoxicity test of Kissmeyer-Nielsen and Kjerrbye and with the National Institutes of Health (NIH) standard technique, using 40 antisera [Kissmeyer-Nielsen and Kjerrbye, 1967].

### Atopy Criteria

The atopy criteria were defined in accordance with our earlier findings of elevated risk of developing LAA symptoms in subjects with family history of allergy and/or a clear sensitization to non-animal environmental allergens [Sjö-

tedt and Willers, 1989; Willers et al., 1992]. Thus, in order to study possible associations between HLA and atopy indicators, the following criteria were used:

1. *Family history of allergy*—including evaluation of allergic symptoms in parents, siblings, and other relatives
2. *Allergy during childhood*—including atopic eczema, rhinoconjunctivitis, and/or asthma.
3. *Sensitization against common environmental allergens*—defined as at least one positive skin prick test reaction  $\geq +1$  relative to the histamine reference
4. *Total serum IgE*— $>100$  kU/L

## Statistics

Prevalence rate ratio (PRR), with both 95% and 99% confidence intervals (CI 95%, 99%), has been calculated in comparison with the population reference group. Fisher's exact *P*-values (two-tailed) have also been calculated and corrected according to the number of antigens. In the statistical calculation, the EPI-Info program (Centers of Disease Control, Atlanta, GA), Version 6.02 has been used.

## RESULTS

The statistical analysis showed that seven HLAs: HLA-A9, -B5, -B12, -B16, -DR4, -DR5, and -DRw6 were associated with symptoms and/or atopy indicators. No other associations between HLA and different atopy criteria, sensitization to laboratory animals, or allergic symptoms were found and therefore are not shown in the tables.

### Symptom Groups and HLAs

The HLA-B5 frequency was elevated in animal test-negative subjects, compared to the population reference group (PRR = 2.5, CI 95% = 1.1–6; Table II). Also for DRw6 the prevalence rate (PR) was elevated in test-negative subjects compared to the reference group (PRR = 2.4, CI 99% = 1.2–4.8). These differences were not statistically significant, however, when corrected for multiple comparisons.

High PR for HLA-B16 was found in the symptom-free group in comparison with the population reference group (PR = 30% vs. PR = 3%), corrected Fisher's exact *P* = 0.005. In the subgroup of LAA asthmatics (*n* = 28), all individuals lacked HLA B16. The prevalence rate of HLA-DR4 in the LAA asthma group (*n* = 28) was elevated compared to the population references (PRR = 1.7, CI 99% = 1.0–2.7). However, when correcting for the number of HLAs studied, the difference is not statistically significant.

### Atopy Indicators and HLAs

HLA-DR5 was elevated, but not statistically significant, in subjects with *parental allergy* (CI 95% = 1.2–6.9; Table III) and also in subjects with *allergy in siblings* (CI 99% = 1.0–8.0). Elevated PR value for HLA DR5 was also found in subjects with *allergy during childhood* (PRR = 2.8, CI 95% = 1.1–7.1).

Subjects sensitized against *environmental allergens* had elevated PR for HLA-A9, but again this difference was not statistically significant (CI 95% = 1.0–3.8). On the contrary, individuals sensitized against environmental allergens, but also to pollen allergens and laboratory animals, totally lacked HLA-B16.

Subjects with *serum IgE*  $>100$  kU/L (*n* = 20) had higher HLA-B12, but this was not statistically significant when corrected for multiple comparisons (PRR = 1.9, CI 95% = 1.1–3.4) and HLA-DRw6 (PRR = 2.2, CI 95% = 1.2–4.3). Subjects with serum IgE  $>100$  kU/L lacked HLA-B16. Individuals with serum IgE  $<10$  kU/L (*n* = 25) had higher HLA-B5 (CI 95% = 1.2–7.4) and HLA-B16. The latter difference was significant when corrected for multiple comparisons (CI 99% = 1.9–39; corrected *p* = 0.015).

### Family and Childhood History of Allergies and Sensitization to Laboratory Animals

Family and childhood history of allergies, reported in the questionnaires, were both associated (PRR = 1.6, CI 95% = 1.0–2.6 and PRR = 1.7, CI 95% = 1.2–2.5, respectively) with sensitization (skin prick test  $\geq +1$ ) to laboratory animals. The PRR was also increased (PRR = 1.6, CI 95% = 1.3–2.0 and PRR = 2.6, CI 95% = 1.4–4.8, respectively) in LAA asthmatic subjects (*n* = 28), compared to the rest (*n* = 91) of the studied population.

## DISCUSSION

Respiratory allergic diseases and possible HLA markers, based on population, twin, and family studies, have shown both increased or decreased PR of specific HLA alleles [Mehra et al., 1992]. In the present study of respiratory allergy and atopy indicators and their possible association with HLA markers, seven HLA alleles suggested a relationship to allergic reactions. After correction for multiple comparisons (number of antigens is a total of 40), only the low or absent HLA-B16 in atopic subjects remained statistically significant.

The elevated HLA-B16 in subjects without symptoms and/or IgE  $<10$  kU/L combined with the complete absence of HLA-B16 in all sensitized and/or serum IgE  $>100$  kU/L, suggest an association between HLA-B16 or an immunoregulatory gene linked to HLA-B16 and the ability to pro-

**TABLE II.** HLA in Relation to Symptoms and Allergic Sensitization in Comparisons With the Population Reference Group

| HLA antigen | Group                | PR (%) | PPR  | CI (95%) | CI (99%) | Fisher's test <i>P</i> |
|-------------|----------------------|--------|------|----------|----------|------------------------|
| B5          | Population reference | 8      | —    | —        | —        | —                      |
|             | Symptom free         | 15     | 1.8  | 0.6–5.4  | 0.4–7.6  | 0.28                   |
|             | Airway symptoms      |        |      |          |          |                        |
|             | Test positive        | 13     | 1.6  | 0.7–4.0  | 0.5–5.4  | 0.4                    |
|             | LAA asthma           | 14     | 1.8  | 0.6–5.2  | 0.4–7.3  | 0.3                    |
|             | Test negative        | 21     | 2.5  | 1.1–6    | 0.82–7.8 | 0.042                  |
| B16         | Population reference | 3      | —    | —        | —        | —                      |
|             | Symptom free         | 30     | 9.1  | 3–28     | 2.1–40   | 0.00013*               |
|             | Airway symptoms      |        |      |          |          |                        |
|             | Test positive        | 0      | —    | —        | —        | —                      |
|             | LAA asthma           | 0      | —    | —        | —        | —                      |
|             | Test negative        | 8      | 2.4  | 0.6–10   | 0.4–16   | 0.36                   |
| DR4         | Population reference | 36     | —    | —        | —        | —                      |
|             | Symptom free         | 33     | 0.91 | 0.5–1.6  | 0.4–2    | 0.83                   |
|             | Airway symptoms      |        |      |          |          |                        |
|             | Test positive        | 47     | 1.3  | 0.89–1.9 | 0.80–2.1 | 0.24                   |
|             | LAA asthma           | 61     | 1.7  | 1.1–2.4  | 1.0–2.7  | 0.032                  |
|             | Test negative        | 36     | 1    | —        | —        | —                      |
| DRw6        | Population reference | 18     | —    | —        | —        | —                      |
|             | Symptom free         | 11     | 0.62 | 0.2–1.9  | 0.14–2.8 | 0.3                    |
|             | Airway symptoms      |        |      |          |          |                        |
|             | Test positive        | 17     | 0.95 | —        | —        | —                      |
|             | LAA asthma           | 14     | 0.8  | 0.3–2.1  | 0.2–2.9  | 0.79                   |
|             | Test negative        | 44     | 2.4  | 1.5–4.1  | 1.2–4.8  | 0.0022                 |

PR, prevalence rate; PPR, prevalence rate ratio; CI, confidence interval.

\**p* = 0.005 corrected for 40 antigens.

duce IgE. This finding is in accordance with the uncommented findings of Anagnostopoulou et al. [1993], who did not find HLA-B16 in any of their patients in a study of 57 cases with chronic bronchitis in contrast to 12/267 (PR = 6%) in the control group.

Sasazuki [1990] reported observations indicating the existence of HLA-linked immunosuppressive (Is) genes in humans, who are low or nonresponders of natural antigens. Our findings that HLA-B16 might "protect" against both allergic symptoms, sensitization (against environmental and animal allergens) and are correlated with serum IgE <10 kU/L might imply association between an Is gene(s) with HLA-B16. The assumption of a "protective" MHC haplotype against ragweed pollen allergy has also been suggested by Blumenthal et al. [1992].

Associations between immune response to well-defined pollen allergens and specific HLA-DR antigens, e.g., ragweed allergen (Amb a V) and elevated frequency of HLA-DR2 (and HLA-B7), have been reported in patients with asthma against ragweed pollen [Blumenthal et al., 1992].

Such findings suggest the existence of immune response (Ir) genes in the HLA complex [Marsh and Huang, 1991].

Our preliminary report of a possible association between HLA-B15 and -DR4 and LAA airway symptoms (especially asthma) was not confirmed in this study, nor was any association with atopy criteria found. However, the trend for elevated PR for HLA-DR4 in the LAA asthmatic group remained, indicating a possibility for linkage of an Ir gene to HLA-DR4. Conflicting results, implying negative association between HLA-DR4 and test-positive mountain-cedar pollinosis, have been reported by Reid et al. [1992]. A subgroup of pollen-sensitized subjects in our study also had low PR of DR4 compared to the population reference group (PR = 17% vs. PR = 36%; *n* = 18), but the difference was not statistically significant.

The ambiguity between questionnaire support for hereditary factors (i.e., family and childhood history of allergies) in LAA and the HLA results might be due to the fact that animal allergens are complex proteins, including major and minor allergens with power to evoke immune response

TABLE III. HLA in Relation to Atopy Criteria Compared to the Population Reference Group

| HLA antigen | Group                                      | PR (%) | PRR | CI (95%) | CI (99%) | Fisher's test P |
|-------------|--------------------------------------------|--------|-----|----------|----------|-----------------|
| A9          | Population reference                       | 16     | —   | —        | —        | —               |
|             | Sensitized against environmental allergens | 32     | 2.0 | 1.0–3.8  | 0.85–4.7 | 0.072           |
| B5          | Population reference                       | 8      | —   | —        | —        | —               |
|             | IgE <10                                    | 24     | 3.0 | 1.2–7.4  | 0.89–9.8 | 0.031           |
| B12         | Population reference                       | 24     | —   | —        | —        | —               |
|             | IgE >100                                   | 45     | 1.9 | 1.1–3.4  | 0.89–4.1 | 0.057           |
| B16         | Population reference                       | 3      | —   | —        | —        | —               |
|             | IgE <10                                    | 28     | 8.6 | 2.7–27   | 1.9–39   | 0.00037*        |
| DR5         | Population reference                       | 8      | —   | —        | —        | —               |
|             | Parental allergy                           | 23     | 2.9 | 1.2–6.9  | 0.90–9.1 | 0.045           |
|             | Allergy in siblings                        | 23     | 2.8 | 1.3–6.2  | 1.0–8.0  | 0.017           |
|             | Childhood allergy                          | 23     | 2.8 | 1.1–7.1  | 0.85–9.5 | 0.037           |
| DRw6        | Population reference                       | 18     | —   | —        | —        | —               |
|             | IgE >100                                   | 40     | 2.2 | 1.2–4.3  | 0.94–5.3 | 0.036           |

PR, prevalence rate; PRR, prevalence rate ratio; CI, confidence interval.  
\*p = 0.015 corrected for 40 antigens.

from different class II molecules, for example. By contrast, purified pollen allergens might associate with particular HLA-DR types [Marsh and Huang, 1991]. Modern techniques, such as polymerase chain reaction (PCR) studies, have also shown considerable polymorphism among the genes in the HLA-DR subregion, including HLA-DR4 [Mehra et al., 1994].

The possible HLA-B16 association with a reduced risk of allergy development has not, according to our knowledge, been reported previously. However, the finding should be cautiously interpreted because of the low PR in the population. This observation needs confirmation in further studies with DNA-based analysis of HLA typing.

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