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Human leucocyte antigens (HLA) and trimellitic anhydride (TMA) immunological lung disease

L. C. Grammer, C. R. Zeiss, P. R. Yarnold and M. A. Shaughnessy

Division of Allergy-Immunology and the Ernest S. Bazley Asthma and Allergic Diseases Center of the Department of Medicine of Northwestern Memorial Hospital and Northwestern University Medical School and the Division of General Internal Medicine, Northwestern University Medical School, Chicago, Illinois, U.S.A.

Occupational immunological lung disease, due to low molecular weight, reactive chemicals such as trimellitic anhydride (TMA), is an emerging health problem. If there were a marker that was highly predictive of the ability of the immune system to recognize TMA as an allergen, better prevention strategies could be employed with at risk individuals. The purpose of this study is to evaluate whether human leucocyte antigen (HLA) class specificity is associated with the development of late respiratory systemic syndrome (LRSS) or asthma due to immunological sensitivity to trimellitic anhydride (TMA). This is a case control study of 17 individuals with LRSS, 12 with asthma and 22 TMA similarly exposed individuals who did not develop LRSS or asthma. Comparing the sensitized individuals (LRSS or asthma) with the non-sensitized individuals (controls), we found no difference in frequency of any HLA antigen. In summary, the lack of association of HLA antigens with LRSS or asthma due to TMA suggests that these will not be useful markers to identify at risk individuals.

Key words: asthma; HLA; occupational disease; trimellitic anhydride.

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Introduction

Occupational immunological lung disease, due to low molecular weight, reactive chemicals such as trimellitic anhydride (TMA), is an emerging health problem (1). TMA is one of several acid anhydrides used as a catalyst in the manufacture of epoxy resins. In spite of low exposure levels, approximately 10% of exposed individuals develop asthma due to TMA. TMA can form covalent bonds with proteins in the airway. TMA conjugated to human proteins such as human serum albumin (HSA) can act as an allergen. The presence of IgE against TMA conjugated to HSA (TM-HSA) is highly associated with asthma due to TMA while IgG against TM-HSA is associated with late respiratory systemic syndrome (LRSS) (2).

At present, early detection of employees with immunological lung disease due to TMA is accomplished through annual surveillance studies of exposed employees. In the current climate of increasing healthcare cost, prevention would, of course, be preferable to surveillance for disease. If there were a marker that was highly predictive of the ability of the immune system to recognize TM-HSA as an allergen, better prevention strategies could be employed with susceptible individuals. Obviously, a potential applicant could not be denied a job because he or she was at high risk for sensitization. However, the applicant could be counseled that the likelihood of sensitization was high; the applicant might decide not to take a job in which there was a high likelihood of sensitization. The goal would be the prevention of TMA immunological lung disease rather than the current strategy of diagnosing the disease and moving the individual to a job that involves less TMA exposure.

It has been reported in several studies of the human immune response to ragweed allergens, *Amb a V, Amb pV* and *Amb t V*, that MHC Class II specificities, HLA-DR2 and HLA-Dw2, are highly associated with the ability to produce IgE against these antigens (3). A low molecular weight chemical causing occupational asthma, toluene diisocyanate (TDI), has been studied by Dr Fabbri and co-workers in Italy. They have reported a significant association between development of TDI asthma and a certain MHC Class II specificity, HLA-DQB1*0503 (4). Dr Baur's group, however, has been unable to confirm the importance of HLA Class II alleles in isocyanate induced asthma (5).

Two groups of investigators have published studies regarding the association of HLA allele frequency and specific IgE against the corresponding acid anhydride-HSA

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Correspondence should be addressed to: Leslie C. Grammer, M.D., Northwestern University Medical School, Department of Medicine, Division of Allergy-Immunology, MC S207, Tarry Building 3-713, 303 E. Chicago Ave., Chicago, IL 60611, U.S.A. Fax: +1 312 908 0205.

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conjugate (6). A British group reported an association between specific IgE against acid anhydride-HSA and the MHC Class II specificity, HLA-DR3. This association was particularly strong for TMA. A Swedish study of workers exposed to two other anhydrides, hexahydrophthlic anhydride (HHPA) and methyltetrahydrophthalic anhydride (MTHPA), reported no association between MHC class specificity and specific antibody in serum (7).

This study is a case control study to evaluate whether HLA MHC class specificity is associated with the development of LRSS or TMA asthma. The question of whether susceptibility to asthma due to low molecular weight sensitizers is predictable is an important question which affects the health of hundreds of thousands of exposed workers worldwide, and studies such as the present one will be the only means to answer this question.

Methods

POTENTIAL STUDY POPULATION

The study population from which we could recruit subjects consists of approximately 80 similarly exposed employees who have been participating in our annual surveillance studies at the manufacturing plant. Approximately 30 employees have developed an immunologically mediated respiratory disease to TMA. About half of those developed IgE against TM-HSA and TMA asthma, and the others have developed LRSS and IgG against TM-HSA.

EXPOSURE CLASSIFICATION

An industrial hygienist at the manufacturing plant has assigned an exposure classification to employees based on a detailed knowledge of each job and on data obtained from TMA personal monitoring of some employees in each exposure classification (8). Exposure categories are 1 to 5, with 1 being the highest exposure and 5 the lowest.

IMMUNOLOGICAL EVALUATION

Blood is drawn annually from each employee to assess IgE and IgG antibody against TM-HSA in serum. These serological tests were performed by radioimmunoassay using standard methodology (9).

CLINICAL EVALUATION

A questionnaire developed jointly by the University of Cincinnati and the National Institute for Occupational Safety and Health to assess occupational immunological lung disease was administered to each employee (10). Any employee with positive symptoms on questionnaire and/or positive antibody results was interviewed, examined and skin tested with TM-HSA by a physician.

The criteria for IgE mediated TMA asthma have been previously published (11). They are: compatible symptoms including one or more of the following—cough, dyspnea, wheeze, chest tightness; physical findings of wheeze or prolonged expiratory phase; obstructive PFTs post-exposure; normal chest film; IgE antibody against TM-HSA demonstrable by *in vitro* or *in vivo* assay. The criteria for LRSS are: compatible symptoms including one or more of the following: cough, fever, dyspnea; physical findings of rales, tachypnea and fever; restrictive PFTs post-exposure; basilar chest infiltrates; IgG antibody against TM-HSA.

CASE SUBJECTS

Case subjects were recruited from those employees who met the criteria for TMA asthma or LRSS as defined above. Informed consent was obtained. There were cases with asthma and/or cases with LRSS.

CONTROL SUBJECTS

Control subjects were recruited from the employees who had not developed IgE or IgG against TM-HSA. Controls had no work related symptoms. Control subjects were matched with case subjects for age, gender, race, smoking, exposure category, and exposure duration.

REFERENT POPULATION

A reference population (Chicago control subjects) of 500 apparently healthy subjects, who had donated blood was included for comparison on the prevalence of the HLA Class I and II antigens.

HLA MHC CLASS TYPING

HLA MHC class typing was performed by the Tissue Typing Laboratory at Northwestern using standard methodology (12).

STATISTICAL ANALYSIS

Comparisons between groups for both categorical and ordered attributes were conducted using the non-parametric statistical procedure, optimal discriminant analysis (ODA) (13–15). When an attribute is dichotomous, the *P*-value obtained via ODA is the same as would be obtained had Fisher's exact test been conducted. Because of the small sample size of the LRSS and asthma groups, alpha correction via the Bonferroni procedure was not performed: effects were deemed statistically significant if the generalized ('per-comparison') Type I error was $P < 0{\cdot}05$.

Results

DEMOGRAPHIC INFORMATION

The cases and controls were not significantly different in terms of any demographic variable studied (Tables 1 and 2).

TABLE 1. Patient attribute comparison: control *vs.* LRSS patients

Attribute	Levels	(Negative for LRSS) Control	(Positive for LRSS) LRSS	*P*-value
Cigarette Smoker	Never	$n=10$	$n=2$	0·33
	Ex-smoker	$n=6$	$n=3$	
	Current	$n=1$	$n=1$	
	Other	$n=12$	$n=10$	
Packs per day	Mean ± SD	0·6 ± 0·8	1·5 ± 1·8	0·36
		$n=17$	$n=6$	
Years smoking	Mean ± SD	9·4 ± 12·7	14·5 ± 13·0	0·38
		$n=17$	$n=6$	
Age (years)	Mean ± SD	37·7 ± 8·0	36·4 ± 7·9	0·40
		$n=31$	$n=16$	
Sex	Male	$n=31$	$n=14$	0·71
	Female	$n=0$	$n=2$	
Race	Caucasian	$n=24$	$n=14$	0·71
	Non-caucasian	$n=6$	$n=2$	
Exposure	Mean ± SD	2·0 ± 0·8	2·0 ± 0·7	0·75
		$n=31$	$n=16$	

Note: Sample sizes vary due to missing data

TABLE 2. Patient attribute comparison: control *vs.* asthma patients

Attribute	Levels	Negative for asthma	Positive for asthma	*P*-value
Cigarette smoker	Never	$n=10$	$n=2$	0.78
	Ex-smoker	$n=6$	$n=3$	
	Current	$n=1$	$n=1$	
	Other	$n=16$	$n=6$	
Packs per day	Mean ± SD	0·9 ± 1·3	0·7 ± 0·7	0·65
		$n=18$	$n=5$	
Years smoking	Mean ± SD	10·8 ± 13·6	10·6 ± 9·9	0·81
		$n=18$	$n=5$	
Age (years)	Mean ± SD	39·0 ± 7·3	32·2 ± 7·7	0·06
		$n=35$	$n=12$	
Sex	Male	$n=34$	$n=12$	0·99
	Female	$n=1$	$n=1$	
Race	Caucasian	$n=29$	$n=9$	0·99
	Non-caucasian	$n=6$	$n=2$	
Exposure	Mean ± SD	2·1 ± 0·8	1·7 ± 0·7	0·09
		$n=35$	$n=12$	

HLA ANTIGEN COMPARISON

Only one statistical comparison was statistically significant (Tables 3, 4 and 5). A greater proportion of the TM control patients were positive for the B7 antigen than were the Chicago control patients (38% *vs.* 18%, respectively, $P<0·029$). Since a total of 123 tests of statistical hypothesis were conducted and then evaluated for statistical significance under the generalized ('per-comparison') criterion of $P<0·05$, a total of $0·05\times123$, or six 'statistically significant' effects would be expected by chance alone. Thus, the finding of only one significant effect reflects less than 17% of the number of significant effects that would be expected by chance alone.

Discussion

Occupational immunological lung disease due to low molecular weight chemicals like TMA is an emerging

TABLE 3. Antigen comparison, control *vs.* LRSS patients

	Controls ($n=22$)		LRSS ($n=17$)		
Antigen	*n* Positive	*n* Negative	*n* Positive	*n* Negative	*P*
A1	4	18	5	12	0·47
A2	12	10	11	6	0·75
A3	4	18	5	12	0·47
A9	3	19	4	13	0·68
A10	2	20	0	17	0·50
A11	5	17	1	16	0·21
A28	2	20	2	15	0·99
A29	2	20	0	17	0·50
AW30	3	19	2	15	0·99
AW31	1	21	1	16	0·99
AW32	2	20	3	14	0·64
Blank	4	18	0	17	0·12
B5	1	21	0	17	0·99
B7	9	13	6	11	0·76
B8	3	19	3	14	0·99
B12	4	18	4	13	0·71
B13	1	21	2	15	0·58
B14	3	19	1	16	0·62
B15	1	21	3	14	0·30
B16	1	21	2	15	0·58
B17	2	20	3	14	0·64
B18	2	20	0	17	0·50
B27	3	19	2	15	0·99
BW35	4	18	3	14	0·99
B40	2	20	4	13	0·38
B21	2	20	0	17	0·50
Blank	6	16	1	16	0·12
DR1	5	17	2	15	0·44
DR3	5	17	2	15	0·44
DR4	8	14	7	10	0·99
DR5	4	18	4	13	0·71
DRW6	6	16	2	15	0·43
DR7	5	17	6	11	0·49
DRW8	0	22	3	14	0·08
Blank	6	16	1	16	0·12

healthcare problem that results in significant morbidity, missed workdays and healthcare costs (16). The current approach is that of periodic surveillance and diagnosing TMA asthma after the individual has already developed disease. If asthma or LRSS are diagnosed at an early stage after the individual has been symptomatic for only a few months, there are no permanent sequelae. Therefore, vigilant surveillance studies do provide appropriate protection of exposed individuals from permanent lung damage.

However, a preferable approach would be that of identifying a marker that is predictive of which individuals are genetically susceptible to recognize TM-protein as an allergen, to develop IgE or IgG against TM-protein and ultimately, TMA induced immunological lung disease. Individuals could be counseled about their high risk of sensitization which could affect their behavior in a variety of positive ways. For instance, if they decided to take the job, they might be more attentive to early signs and symptoms of occupational immunological lung disease. A good candidate for such a predictive marker is human leucocyte antigen (HLA) major histocompatibility complex (MHC) Class II specificity. This is because immune responsiveness of a given individual to a given allergen is highly dependent upon the ability of that allergen to bind to HLA Class II molecules on the surface of that individual's T lymphocytes. If an individual's HLA Class II molecules cannot bind the allergen, it cannot be recognized by that individual's immune system. Conversely, an individual's immune system can recognize an allergen that will bind to its HLA Class II molecules (17).

TABLE 4. Antigen compresions: control *vs*. asthma patients

	Controls ($n=22$)		Asthma ($n=12$)		
Antigen	*n* Positive	*n* Negative	*n* Positive	*n* Negative	$P<$
A1	4	18	5	7	0·23
A2	12	10	6	6	0·99
A3	4	18	3	9	0·68
A9	3	19	2	10	0·99
A10	2	20	1	11	0·99
A11	5	17	1	11	0·39
A28	2	20	0	12	0·53
A29	2	20	0	12	0·53
AW30	3	19	1	11	0·99
AW31	1	21	1	11	0·99
AW32	2	20	2	10	0·61
Blank	4	18	2	10	0·99
B5	1	21	1	11	0·99
B7	9	13	2	10	0·26
B8	3	19	3	9	0·65
B12	4	18	3	9	0·68
B13	1	21	2	10	0·28
B14	3	19	0	12	0·54
B15	1	21	1	11	0·99
B16	1	21	2	10	0·28
B17	2	20	2	10	0·61
B18	2	20	1	11	0·99
B27	3	19	2	10	0·99
BW35	4	18	3	9	0·68
B40	2	20	4	8	0·16
B21	2	20	0	12	0·53
Blank	6	16	2	10	0·69
DR1	5	17	2	10	0·99
DR2	5	17	5	7	0·28
DR3	5	17	2	10	0·99
DR4	8	14	5	7	0·99
DR5	4	18	3	9	0·68
DRW6	6	16	0	12	0·07
DR7	5	17	4	8	0·69
DRW8	0	22	2	10	0·12
Blank	6	16	2	10	0·69

To address this issue, we conducted a case controlled study to compare individuals with TMA asthma to appropriately matched controls in terms of HLA MHC class specificity. This study design has been used successfully by others (4–7). IgE responsiveness against other allergens, *Amb a V, Amb p V* and *Amb t V*, has been found to be highly associated with MHC Class II specificities (3).

Some investigators have found HLA associations with asthma due to isocyanate and acid anhydrides while others have not (4–7). We did not find an association in this study. Other investigators have reported HLA-DR3 as an association. There was no difference in our study of HLA-DR3 in sensitized and non-sensitized individuals. We have followed these controls for more than a decade to be certain that they would not develop OILD with additional exposure. Our findings are similar to those of Nielson and colleagues who also could find no HLA MHC class association.

It should be noted that our small, imbalanced samples yielded low statistical power (10–45%) for detecting differences between groups. This could have been a problem if, for instance, the individuals with TMA asthma had a particular HLA allele at a three-fold higher frequency than controls, but that difference was not statistically significant. This would obviously raise the issue of the power of the study. In our study, the sensitized individuals have

TABLE 5. Antigen compresions: TM control *vs.* Chicago control patients

	TM Controls ($n=24$)		Chicago Controls ($n=500$)		
Antigen	*n* Positive	*n* Negative	*n* Positive	*n* Negative	*P*
A1	4	20	125	375	0·47
A2	12	11	230	270	0·54
A3	4	20	120	380	0·63
A9	4	20	90	410	0·99
A10	2	22	75	425	0·56
A11	5	17	65	435	0·35
A28	3	21	50	450	0·73
A29	2	22	35	465	0·69
AW30	3	21	25	475	0·14
AW31	1	23	30	470	0·99
AW32	3	21	35	465	0·41
Blank	4	20			
B5	1	23	55	445	0·50
B7	9	15	90	410	0·029
B8	3	21	100	400	0·60
B12	5	19	120	380	0·99
B13	1	23	25	475	0·99
B14	3	21	49	451	0·73
B15	1	23	49	451	0·72
B16	2	22	50	450	0·99
B17	2	22	55	445	0·99
B18	2	22	50	450	0·99
B27	3	21	35	465	0·41
BW35	4	20	80	420	0·99
B40	3	21	60	440	0·99
B21	2	22	35	465	0·69
Blank	6	18			
DR1	5	19	99	401	0·80
DR2	5	19	126	374	0·82
DR3	6	18	115	385	0·81
DR4	8	16	150	350	0·83
DR5	4	20	95	405	0·99
DRW6	7	17	135	365	0·82
DR7	5	19	114	386	0·99
DRW8	2	22	48	452	0·99
DRW10	0	24	10	490	0·99
Blank	6	18	150	350	0·82

essentially the same HLA allele frequency as the controls. Therefore, we did not believe that power was an issue. In order to verify this, we assessed whether statistically significant effects would emerge if we had large samples. To do this, we repeated the analyses after first multiplying the sample size by 10, an order of magnitude. For this hypothetical sample, there were more than three times the number of statistically significant effects than would be expected by chance alone. However, none of the statistically significant effects was judged to be clinically significant as defined by a relative risk >2.

In summary, we tested the hypothesis that the specificity of IgE or IgG and associated asthma or LRSS was associated with HLA MHC allele frequency. The lack of association suggests that these will not be useful markers to identify at risk individuals. Further studies with anhydrides and other agents will be important to future define the utility of HLA alleles in susceptibility to occupational immunological lung disease.

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