

HLA associations with occupational sensitization to rat lipocalin allergens: A model for other animal allergies?

Hayley Jeal, BSc,^a Adrian Draper, MRCP,^a Meinir Jones, PhD,^a Jessica Harris, MSc,^a Ken Welsh, PhD,^b Anthony Newman Taylor, FRCP,^a and Paul Cullinan, MD^a *London, United Kingdom*

Background: Laboratory animal allergy is a common occupational health problem affecting between 11% and 44% of exposed researchers. Allergy to rats and mice is most common, probably because these are the animals most frequently used. **Objective:** We hypothesized that HLA class II molecules, involved in the presentation of allergen to the T cell and likely candidates for controlling the immune response, might be associated with sensitization to rat urinary proteins among laboratory animal handlers.

Methods: We undertook a cross-sectional study of 741 employees at 6 pharmaceutical sites across the United Kingdom who had contact at work with laboratory rats. In all, 109 cases with specific sensitization to rat proteins and 397 referents were HLA-typed for DRB1 and DQB1 loci. Amino acid analyses of significantly associated HLA molecules were carried out.

Results: HLA-DR7 was associated with sensitization (odds ratio [OR], 1.82; CI, 1.12-2.97), respiratory symptoms at work (OR, 2.96; CI, 1.64-5.37) and, most strongly, sensitization with symptoms (OR, 3.81; CI, 1.90-7.65). HLA-DR3 was protective against sensitization (OR, 0.55; CI, 0.31-0.97). Amino acid analyses of these 2 molecules indicated a biologically plausible explanation for the associations.

Conclusion: HLA phenotype is an important determinant of individual susceptibility to sensitization and asthma among laboratory animal workers. Similar mechanisms might apply in other animal allergies. (*J Allergy Clin Immunol* 2003;111:795-9.)

Key words: Lipocalin, Rat n 1, occupational allergy, HLA, animal allergy

Rodents, particularly rats, are a frequent cause of allergy and asthma among laboratory animal workers. It is estimated that between 12,000 and 17,000 researchers in the United Kingdom (UK) are currently working with laboratory animals.¹ Epidemiologic studies of laboratory animal allergy reveal a range of prevalence values of 11% to 44%.^{1a} The major rat allergen, Rat n 1, is excreted in urine and has been characterized as a 17-kd protein.² Like several other animal allergens, including the major

Abbreviations used

OR: Odds ratio

UK: United Kingdom

mouse urinary protein, Mus m 1, and the cow allergen Bos d 2, Rat n 1 belongs to a group of proteins called *lipocalins*, which function as carrier molecules in the transport of hydrophobic ligands.³

HLA class II antigens are involved in the presentation of epitopes of foreign proteins at the antigen-presenting cell surface and are important in the development of a hypersensitivity response. Several HLA associations with occupational low-molecular-weight chemical sensitizers have been demonstrated,⁴⁻⁶ but there are few studies with similar strengths of association for occupational proteins. It is possible that multiple epitopes derived from larger allergen proteins are presented by different HLA molecules, diluting any single HLA association.

Given that Rat n 1 is a relatively low-molecular-weight protein with just 5 identified T-cell epitopes,⁷ we hypothesized that the risk of developing specific IgE antibody to rat urinary protein among those encountering rats in their work would be associated with restricted HLA alleles. On the basis of our previous work in the platinum-refining industry,⁵ we also hypothesized that the strength of associations would be greater in those with least exposure to rat proteins. Here we report a cross-sectional study of pharmaceutical company employees in the United Kingdom; the investigation was designed to test these hypotheses.

METHODS

Between September 1999 and May 2001 we carried out cross-sectional surveys of employees at 6 pharmaceutical companies in the United Kingdom. The ethics committee of Royal Brompton Hospital/NHLI approved the study, and written informed consent was obtained from all volunteers. Any current employee identified from health surveillance registers as having had at least 1 month's contact with rat proteins was considered eligible. Each such employee was asked to complete a questionnaire, undergo skin prick tests, and provide a blood sample.

Exposure assessment

In addition to capturing demographic details, the questionnaire was used to categorize prior exposure to rat proteins. Details of current and previous employment, the maximum frequency of entering

From ^athe Department of Occupational and Environmental Medicine and ^bthe Department of Interstitial Lung Disease, Imperial College (NHLI).

This work was funded by the National Asthma Campaign.

Received for publication November 7, 2002; revised November 28, 2002; accepted for publication December 9, 2002.

Reprint requests: Meinir G Jones, PhD, Department of Occupational and Environmental Medicine, Faculty of Medicine, Imperial College (NHLI), 1B Manresa Rd, London SW3 6LR, United Kingdom.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.176

TABLE I. Distribution of demographic and exposure variables by case status

Variable	Sensitized employees (n = 109)	Nonsensitized employees (n = 397)	P value
Male: n (%)	61 (56)	215 (54)	.74
Age (y): median (range)	33 (18-61)	34 (18-65)	.20
White: n(%)	102 (94)	377 (95)	.57
Ever smoked: n (%)	36 (33)	137 (35)	.73
No. of years exposed to rats: median (range)	9 (0.2-30)	9 (0.1-42)	.83
Maximum frequency of entering animal housing facility: n (%) daily	97 (89)	284 (72)	<.001
Personal protection first used: n (%)			.41
Powered helmet or ventilated cabinet	3 (3)	23 (6)	
Mask	37 (34)	139 (35)	
None	69 (63)	235 (59)	
Highest exposed previous job			.83
Low: office/maintenance worker	6 (6)	41 (10)	
Medium: scientist	86 (79)	270 (68)	
High: animal technician/cage cleaner	17 (16)	86 (22)	
Site			.01
1	17 (16)	53 (13)	
2	27 (25)	101 (25)	
3	13 (12)	109 (27)	
4	22 (20)	69 (17)	
5	14 (13)	25 (6)	
6	16 (15)	40 (10)	
Atopy: n (%)	78 (72)	144 (36)	<.001
Work-related chest symptoms: n (%)	45 (42)	12 (3)	<.001

an animal housing facility, and the maximum number of rats handled in a single day were recorded. An estimate of duration of contact with rat proteins was derived from the dates of the first and last handlings of rats. Employees were classified into groups of exposure according to the maximum frequency of ever entering an animal housing facility (daily or less often than daily) and according to the highest previous job exposure (office/maintenance worker = low; scientist = medium; animal technician/cage cleaner = high).⁸ They were also classified into 3 groups according to the type of personal respiratory protection used when they first worked with rat proteins (powered helmets, masks, or no protection).

Symptoms

The same questionnaire was used to inquire into work-related chest symptoms, defined as those occurring at work and/or improving when away from work.

Skin prick testing

Skin prick tests were undertaken with physiologic saline solution, histamine, grass pollen, cat, *Dermatophagoides pteronyssinus*, guinea pig, rabbit, dog (Allergopharma, Reinbek, Germany), latex (Stallergenes, Antony Cedex, France), and rat and mouse urinary extracts (Royal Brompton Hospital, London, United Kingdom). Wheal sizes were recorded after 10 minutes by measuring the mean of their longest and midpoint orthogonal internal diameters; a positive response was one with a mean wheal diameter at least 3 mm greater than obtained in response to saline solution. Quality control was achieved by ensuring that the coefficient of variability of wheal sizes for each tester was less than 20% for 16 repeated positive histamine controls.⁹ Atopy was defined as a positive response to tests with 1 or more extracts of cat, grass pollen, or *D pteronyssinus*.

Measurement of specific IgE

Specific IgE to an extract of rat urine was measured through use of a RAST. Briefly, 3 mg of lyophilized rat urine (collected from male Sprague Dawley rats) was coupled to 300 mg of activated discs

by the method of Ceska et al.¹⁰ Sera (50 μ L) were incubated at room temperature overnight with discs in duplicate. Bound allergen was detected by adding 50 μ L ¹²⁵I anti-IgE (Pharmacia and Upjohn, Milton Keynes, United Kingdom). After 16 hours of incubation at room temperature, the discs were washed and the radioactivity was counted with a gamma scintillation counter. Each result is expressed as the percentage binding of the total ¹²⁵I anti-IgE added.

Genomic DNA preparation

Genomic DNA was extracted from peripheral blood leukocytes through use of a high-salt technique with minor modifications, as previously described.¹¹ HLA-DRB1 and HLA-DQB1 were typed without knowledge of individual sensitization status through use of PCR with sequence-specific primers, as previously described.¹² All PCR reactions were performed in a final reaction volume of 13 μ L, excluding 10 μ L of light mineral oil. The PCR reaction mixture consisted of the following: 67 mmol/L Tris base, pH 8.8; 16.6 mmol/L NH_4SO_4 ; 2 mmol/L MgCl_2 ; 0.01% (v/v) Tween 20; 200 μ mol/L each of deoxyadenosine, deoxyguanosine, deoxyadenosine, and deoxythymidine triphosphate; 1 to 4 μ mol/L of each allele-specific primer; 0.1 μ mol/L of each control primer; between 0.1 and 0.001 μ g DNA; and 0.1875 U Taq DNA polymerase. The sequence of addition of buffer mixture to specific primers was described previously.¹² PCR amplifications were carried out in an MJ Research PTC-192V machine (Genetic Research Instrumentation Ltd, Dunmow, United Kingdom). The cycling parameters were as follows: 1 minute at 96°C; 5 cycles of 25 seconds at 96°C and 45 seconds at 72°C; 21 cycles of 25 seconds at 96°C, 50 seconds at 65°C, and 45 seconds at 72°C; and 4 cycles of 25 seconds at 96°C, 60 seconds at 55°C, and 120 seconds at 72°C. The plates were sealed and the lid of the thermocycler was adjusted to ensure firm and even pressure on the plate within the machine. The PCR products were electrophoresed (200 V for 15 minutes) in 1.5% agarose gels containing 0.5 μ g/mL ethidium bromide after the addition of 10 μ L of Orange G loading buffer to each well of the PCR plate; results were visualized under UV radiation and then photographed.

TABLE II. HLA prevalences in cases, referents, and UK blood donors. Crude and adjusted ORs for sensitized and non-sensitized participants

HLA	Sensitized cases (n = 109)		Nonsensitized referents (n = 397)		UK donors (n = 603): percent	OR	95% CI	Adjusted OR*	95% CI
	Number	Percent	Number	Percent					
DR1	23	21.1	89	22.4	19.3	0.93	0.55 - 1.55	1.10	0.63 - 1.95
DR15	27	24.8	106	26.7	27.9	0.90	0.55 - 1.57	0.97	0.57 - 1.66
DR16	1	0.9	3	0.8	1.2	1.21	0.13 - 11.81	1.31	0.10 - 16.5
DR3	20	18.4	114	28.7	26.1	0.56	0.33 - 0.95	0.55	0.31 - 0.97
DR4	39	35.8	141	35.5	35.0	1.01	0.65 - 1.57	0.91	0.56 - 1.48
DR11	22	20.2	47	11.8	12.5	1.88	1.08 - 3.29	1.66	0.90 - 3.08
DR12	1	0.9	5	1.3	2.7	0.73	0.08 - 6.28	0.60	0.06 - 5.84
DR13	20	18.4	78	19.7	19.3	0.92	0.53 - 1.58	0.78	0.43 - 1.41
DR14	4	3.7	16	4.0	4.7	0.91	0.30 - 2.77	0.89	0.27 - 2.98
DR7	43	39.5	113	28.5	26.7	1.64	1.05 - 2.55	1.82	1.12 - 2.97
DR8	3	2.8	10	2.5	4.8	1.10	0.30 - 4.05	0.70	0.17 - 2.84
DR9	2	1.8	17	4.3	2.5	0.42	0.10 - 1.84	0.59	0.12 - 2.93
DR10	1	0.9	3	0.8	2.3	1.22	0.13 - 11.81	0.82	0.07 - 9.03
DQ2	50	45.9	183	46.1	49.0	0.99	0.65 - 1.52	1.00	0.63 - 1.58
DQ3	73	67.0	221	55.7	59.2	1.61	1.03 - 2.52	1.43	0.88 - 2.33
DQ4	4	3.7	10	2.5	0.1	1.47	0.45 - 4.80	0.91	0.26 - 3.22
DQ5	25	22.9	112	28.2	29.6	0.76	0.46 - 1.25	0.86	0.50 - 1.49
DQ6	40	36.7	166	41.8	37.8	0.81	0.52 - 1.25	0.74	0.46 - 1.20

Boldface indicates significant associations.

*Adjusted for atopy, maximum frequency of entering animal housing facility, and site.

Subjects

In all, 741 (66%) of 1121 eligible current employees participated in the study; blood samples were collected from 718 (97%). A case of sensitization was defined as the presence of a positive skin prick test result with the extract of rat urine, a RAST binding of $\geq 2\%$, or both; in this way, 115 cases (16%) were identified, all but 6 of which were successfully HLA-typed. A sample of 397 (65%) nonsensitized, referent individuals were similarly typed. Comparisons were also made with HLA distributions among 603 potential UK transplant donors.^{12,13}

The characteristics of the HLA-typed rat workers are shown in Table I. The case and referent populations were similar in their distributions of age, sex, ethnic origin, smoking habit, duration of exposure to rat proteins, and type of personal respiratory protection used when they first worked with rats.

Statistical analysis

Thirteen HLA-DR β 1 loci and 5 HLA-DQ β 1 loci were examined. Univariate distributions were compared through use of *t* tests, Mann-Whitney tests, χ^2 tests, or tests for trend, as appropriate. Differences between sensitized and nonsensitized subjects and symptomatic and nonsymptomatic subjects were analyzed through use of logistic regression, results being expressed as odds ratios (ORs) and 95% CIs. Associations between HLA-DR β 1 and HLA-DQ β 1 loci and sensitization or symptom status were adjusted according to factors that were independently associated with sensitization or symptoms, as appropriate. All analyses were done through use of SAS (SAS Institute Inc, Cary, NC) and Stata (Stata Corporation, College Station, Tex) statistical software.

RESULTS

At univariate analysis, cases were twice as likely to be atopic as referents (Table I). They were also more likely to have entered an animal housing facility on a daily basis, but they were less likely to have worked in the highest expo-

sure job category. Rates of sensitization differed among the 6 sites. The prevalence of work-related chest symptoms was much higher among those sensitized (42% vs 3%).

The distributions of HLA genotypes by case status are shown in Table II; shown alongside, for comparison purposes, are the distributions of each HLA-DR β 1 and -DQ β 1 locus found in the donor population. Unadjusted ORs were increased for HLA-DR β 1*07, HLA-DR β 1*11, and HLA-DQ β 1*03 and were decreased for HLA-DR β 1*03. After adjustment for atopy, exposure, and site of employment (Table III), a significant positive association between HLA-DR β 1*07 and case status remained (OR, 1.82; 95% CI, 1.12-2.97); HLA-DR β 1*03 remained inversely associated with sensitization (adjusted OR, 0.55; 95% CI, 0.31-0.97).

HLA-DR β 1*07 was significantly associated with the presence of work-related chest symptoms (OR, 2.96; 95% CI, 1.64-5.37; *P* < .001) independently of atopic status and maximum frequency of entering an animal housing facility (Table III). Moreover, in comparison with what was seen in unsensitized, asymptomatic employees, the same allele was strongly associated with the reporting of work-related chest symptoms in those with rat protein sensitization (OR, 3.81; 95% CI, 1.90-7.65; *P* < .001). There were no significant associations between HLA-DR β 1*03 and symptoms.

Sensitized individuals were more likely to be HLA-DR β 1*07-positive or HLA-DR β 1*03-negative (89%) than were nonsensitized individuals (77%; *P* = .005). Similarly sensitized individuals with work-related chest symptoms were almost twice as likely to be HLA-DR β 1*07-positive (56%) as those with neither characteristic (28%; *P* $\leq$.001).

TABLE III. Results from logistic regression models

	Adjusted* OR (95% CI) by outcome		
	Sensitization	Work-related chest symptoms	Sensitization and work-related chest symptoms
DRβ1*07	1.82 (1.12-2.97)	2.96 (1.64-5.37)	3.81 (1.90-7.65)
Atopy	4.79 (2.94-7.81)	3.60 (1.92-6.76)	5.03 (2.41-10.47)
Daily work in animal housing facility	3.76 (1.91-7.39)	2.27 (1.00-5.04)	5.45 (1.81-16.45)
DRβ1*03	0.55 (0.31-0.97)	0.71 (0.35-1.45)	0.57 (0.24-1.32)
Atopy	4.63 (2.85-7.52)	3.49 (1.88-6.49)	4.93 (2.44-10.08)
Daily work in animal housing facility	3.61 (1.85-7.05)	2.10 (0.95-4.64)	4.54 (1.55-13.35)

*Adjusted for other factors in the table and site.

The risk of sensitization associated with HLA alleles varied with different levels of exposure, but the differences were seldom statistically significant. The associations between HLA-DRβ1*07 and sensitization varied across exposure categories, but the pattern was inconsistent (ORs: low, 2.15; medium, 1.88; high, 3.86) and not statistically significant (interaction test *P* value, .76). However, the association was more marked among those who entered the animal housing facility at least daily (OR, 2.58; interaction test *P* value, .03). There was no evidence of any modification by exposure of the association between HLA-DRβ1*07 and work-related chest symptoms (*P* = .10) or both work-related chest symptoms and sensitization (*P* = .88). For none of the exposure measurements was there any evidence of interaction between HLA-DRβ1*03 and any of the outcomes.

DISCUSSION

To date, this is the largest study of HLA associations with specific symptoms and sensitization to a single species of laboratory animal. Sensitized individuals were twice as likely to be HLA-DRβ1*07-positive and half as likely to be HLA-DRβ1*03-positive. These associations were independent of age, sex, and broad indicators of exposure. HLA-DRβ1*07 was also, and more strongly, independently associated with work-related chest symptoms. Among employees similarly exposed to rats in their work, those who develop symptomatic sensitization to rat urinary protein are nearly 4 times as likely to be HLA-DRβ1*07-positive as those who remain unsensitized and asymptomatic. This risk estimate was lower than those associated with atopy, or a crude measure of exposure. We did not, however, find any clear evidence that the risks associated with HLA type were modified by intensity of allergen exposure. These figures suggest that approximately 40% of occupational asthma in this population can be attributed to an HLA-DRβ1*07 phenotype; in comparison, attributable proportions for atopy and daily work in an animal housing facility are 58% and 74%, respectively.

Allergy and asthma caused by agents inhaled at work provide an unusual opportunity to investigate the influence of genetic susceptibility on the development of IgE-associated disease. Relative to the investigation of candidate gene associations with allergy and asthma in the community, the population at risk can be clearly enumerated, allowing all prevalent cases to be identified. Occu-

pational case definitions are precise, and because it is possible more accurately to estimate exposure to causative occupational allergens, comparisons can be made between sensitized and nonsensitized individuals with similar exposures. For the same reasons, the study of gene-environment interactions is facilitated.

There have been very few studies of HLA associations with laboratory animal allergy, and there have been none relating to a single species. In reports of sensitized employees of research laboratories in a Swedish university, statistically significant associations with HLA-B15, HLA-DR4, and (inversely) HLA-B16 have been reported.^{14,15} The phenotype used in these studies was not specific, and it is not clear whether the findings were reproducible. We have previously reported an association between HLA-DR3 and the risk of occupational sensitization to ammonium hexachloroplatinate; the strength of the association was greater among those less exposed.⁵ We did not reproduce this finding here.

Our findings should be interpreted with regard to the limitations of and potential sources of bias in our study. Of a cross-sectional nature, recruitment was hampered by the economic climate in the UK pharmaceutical industry at a time when multiple company mergers were underway. With a response rate of 66%, we cannot exclude selection bias and probably undersampled sensitized employees. However, it is unlikely that HLA phenotype influenced participation. Our case definition was specific and incorporated more than one immunologic test result, decreasing the likelihood of misclassification of outcome. The median duration of "exposure" to rats was 9 years, and undoubtedly there will have been a healthy survivor effect among employees with a longer period of employment in this type of work. The exposure assessment relied on recall of individuals, and those with known allergy to rats might recall exposures more clearly than nonsensitized individuals. Nevertheless, these findings are likely to reflect a true association; the distribution of HLA types among nonsensitized individuals was very similar to that seen in an unrelated UK tissue donor population and can be considered representative of the general population.

The study did not have a specific prior hypothesis concerning the association of *specific* HLA alleles with IgE antibody to rat urinary protein, and we have not corrected our levels of statistical significance for multiple tests.¹⁶ The findings should be considered as hypothesis-generating and should be tested in a separate population.

Nevertheless, they have a biological plausibility. Analyses of HLA-DR β 1*07 and HLA-DR β 1*03 molecules reveal that they possess certain unique amino acids within the molecule. HLA-DR β 1*07, associated with both rat sensitization and symptoms, has 3 unique amino acids: lysine at position 14, glutamine at position 25, and leucine at position 30. Leucine, a small hydrophobic amino acid, might be of particular importance at this position, which is located on the floor within pocket 7 of the HLA-DR β 1*07 peptide binding groove and is potentially a peptide contact residue. The lipocalin Rat n 1 is known to bind to hydrophobic ligands, and it is plausible that Rat n 1 binds with high affinity to leucine at position 30. HLA-DR β 1*07 is the only UK common allele to have a hydrophobic amino acid at this position.

HLA-DR β 1*03, which in this study was associated with a protective effect as regards sensitization to rat allergen, has 2 unique amino acids: arginine at position 74 and asparagine at position 77. Arginine at position 74 is located on the α helix within pocket 4 of the peptide binding groove and is the only common UK allele with a positively charged residue at this position. Position 74 is also potentially a peptide and/or T-cell contact residue.

Comparison of the amino acid sequences of HLA-DR β 1*07 and HLA-DR β 1*03 revealed differences in their amino acid sequences. Of particular interest, of 7 residues in which hydrophobicity differed between the molecules, 6 were hydrophobic in HLA-DR β 1*07 and hydrophilic in HLA-DR β 1*03. Pocket 9 of the peptide binding groove has 3 polymorphic residues (positions 9, 37, and 57) that can vary between being hydrophilic and being hydrophobic. In HLA-DR β 1*07 all are hydrophobic, whereas in HLA-DR β 1*03 all are hydrophilic. Position 57 is located on the α helix with the side chains pointing in toward the peptide binding groove, and it is potentially a peptide and/or T-cell receptor contact residue. Intriguingly, substitutions at the equivalent position 57 of the HLA-DR β 1 molecule have been implicated in other occupational diseases—namely, HLA-DQ β 1 position 57 in isocyanate-induced asthma and HLA-DP β 1 position 55 in chronic beryllium disease. HLA-DP β 1 position 55 has also been associated with susceptibility and resistance to type 1 diabetes.^{17,18} Rat n 1 is known to bind hydrophobic ligands, and pocket 9 of the peptide binding groove, made up of predominantly hydrophobic residues in HLA-DR β 1*07, could increase binding affinity for Rat n 1, while the hydrophilic residues of HLA-DR β 1*03 could have a negative effect on binding.

The HLA associations identified within this study might have wider implications for other major animal allergens. In common with rat, the major mouse urinary protein Mus m1, rabbit 18- and 21-kd allergens, bovine allergen Bos d 2, canine allergens Can f 1 and Can f 2, horse allergens Equ c 1 and Equ c 2, and cockroach allergen Bla g 4 all belong to the lipocalin family.³ Lipocalins share structurally conserved regions. Our recent epitope-mapping studies of Rat n 1 have identified T-cell epitopes that overlap with those identified in Bos d 2, the latter being located predominantly in the structurally conserved

regions of the molecule.¹⁹ In the light of the similarities between lipocalin animal allergens and the restricted number of T-cell epitopes identified in both Rat n 1 and Bos d 2, it is conceivable that similar HLA associations will be found with the other major animal lipocalin allergens.

We thank the management and staff at each survey site for allowing the department to undertake surveys on their premises, and we thank all of the individuals who carried out the fieldwork. We also thank the National Asthma Campaign for funding this work.

REFERENCES

- Draper A, Cullinan P, Newman Taylor A. Estimating the incidence of occupational asthma and rhinitis due to laboratory animal allergens during 1999-2000. *Occup Environ Med*. In press 2002.
- Sjöstedt L, Willers S, Orbaek P. Laboratory animal allergy: a review. *Indoor Environ* 1995;4:67-79.
- Longbottom JL. Characterisation of allergies from the urines of experimental animals. In: Kerr JW, editor. *Proceedings of the Eleventh International Congress for Allergology and Clinical Immunology*. London: The Macmillan Press; 1983. p. 525-9.
- Flower DR. The lipocalin protein family: structure and function. *Biochem J* 1996;318(Pt 1):1-14.
- Mapp CE, Balboni A, Baricordi R, Fabbri LM. Human leukocyte antigen associations in occupational asthma induced by isocyanates. *Am J Respir Crit Care Med* 1997;156:S139-S143.
- Newman Taylor AJ, Cullinan P, Lympay PA, Harris JM, Dowdeswell RJ, du Bois RM. Interaction of HLA phenotype and exposure intensity in sensitization to complex platinum salts. *Am J Respir Crit Care Med* 1999;160:435-8.
- Young RP, Barker RD, Pile KD, Cookson WO, Taylor AJ. The association of HLA-DR3 with specific IgE to inhaled acid anhydrides. *Am J Respir Crit Care Med* 1995;151:219-21.
- Jones MG, Jeal H, Newman Taylor AJ. Determining T cell epitopes of rat n1.02 and HLA association in individuals with laboratory animal allergy. *Am J Respir Crit Care Med* 2000;161:A594.
- Gordon S, Tee RD, Nieuwenhuijsen MJ, Lowson D, Harris J, Newman Taylor AJ. Measurement of airborne rat urinary allergen in an epidemiological study. *Clin Exp Allergy* 1994;24:1070-7.
- Skin tests used in type I allergy testing. Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989;44 Suppl 10:1-59.
- Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1-9.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Bunce M, O'Neill CM, Barnardo MC, Krausa P, Browning MJ, Morris PJ, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens* 1995;46:355-67.
- Bunce M, Welsh KI. Rapid DNA typing for HLA-C using sequence-specific primers (PCR-SSP): identification of serological and non-serologically defined HLA-C alleles including several new alleles. *Tissue Antigens* 1994;43:7-17.
- Sjöstedt L, Willers S, Orbaek P. Human leukocyte antigens in occupational allergy: a possible protective effect of HLA-B16 in laboratory animal allergy. *Am J Ind Med* 1996;30:415-20.
- Low B, Sjöstedt L, Willers S. Laboratory animal allergy—possible association with HLA B15 and DR4. *Tissue Antigens* 1988;31:224-6.
- Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43-6.
- Nepom GT, Erlich H. MHC class-II molecules and autoimmunity. *Annu Rev Immunol* 1991;9:493-525.
- Todd JA, Acha-Orbea H, Bell JI, Chao N, Fronck Z, Jacob CO, et al. A molecular basis for MHC class II-associated autoimmunity. *Science* 1988;240:1003-9.
- Jeal H, Jones M, Cullinan P, Newman Taylor AJ. T cell response to rat urinary protein in sensitised and non sensitised individuals and a non exposed control group. *J Allergy Clin Immunol* 2002;109:S178.