

ORIGINAL PAPER

Mouse allergen-specific immunoglobulin G4 and risk of mouse skin test sensitivity

E. C. Matsui*, G. B. Diette*, E. J. M. Krop†, R. C. Aalberse‡, A. L. Smith§ and P. A. Eggleston*

*Johns Hopkins University, Baltimore, MD, USA, †University of Amsterdam, Amsterdam, The Netherlands, ‡Sanquin CLB Blood Supply Foundation and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands and §The Jackson Laboratory, Bar Harbor, ME, USA

Clinical and Experimental Allergy

Summary

Background High serum levels of cat-specific IgG and IgG4 are associated with protection against allergic sensitization to cat, but whether this association applies to other animal allergens remains unclear.

Objective To determine if high levels of mouse-specific IgG and IgG4 are associated with a decreased risk of mouse skin test sensitivity.

Methods Two hundred and sixty workers of a mouse facility underwent skin prick testing and completed a questionnaire. Serum levels of mouse-specific IgG and IgG4 were quantified by solid-phase antigen binding assays. Room air samples were collected and airborne Mus m 1 was quantified by ELISA.

Results Forty-nine participants had a positive skin prick test to mouse. Mouse-specific IgG was detected in 219 (84%) participants and IgG4 was detected in 72 (28%) participants. A detectable mouse-specific IgG4 level was associated with an increased risk of mouse skin test sensitivity (odds ratios (OR) 6.4, 95% confidence intervals (CI) 3.3–12.4). Mouse-specific IgG and IgG4 were both positively correlated with mouse allergen exposure ($r_s = 0.31$, $P = 0.0001$, and $r_s = 0.27$, $P = 0.0006$, respectively). The odds of skin test sensitivity peaked at moderate levels of IgG4, but decreased at the highest levels of mouse-specific IgG4. In contrast, the odds of skin test sensitivity increased monotonically with IgG levels.

Conclusions A detectable level of mouse-specific IgG4 is associated with an increased risk of skin test sensitivity to mouse. However, the highest IgG4 levels appear to be associated with an attenuated risk of mouse skin test sensitivity, suggesting that induction of high levels of IgG4 through natural exposure may protect against the development of allergic sensitization.

Keywords allergen exposure, allergen-specific IgG, allergen-specific IgG4, mouse allergen

Submitted 30 November 2004; revised 3 April 2006; accepted 19 May 2006

Correspondence:

Elizabeth C. Matsui, The Johns Hopkins Hospital, CMSC 1102, 600 N. Wolfe St., Baltimore, MD 21287, USA.
E-mail: ematsui@jhmi.edu

Introduction

Mouse allergy is a common occupational disease and appears to play an important role in childhood asthma in the inner-city [1, 2]. As many as 20% of laboratory mouse workers [3] and 18% of inner-city children with asthma have evidence of allergic sensitization to mouse. Half of sensitized workers report mouse-related symptoms, and one-third report mouse-related lower respiratory symp-

toms. As a result, there have been efforts to reduce mouse allergen exposure in occupational settings [4–6], with the hope that exposure reduction will result in a decreased risk of developing IgE-sensitization to mouse. However, the potential impact of exposure reduction on risk of allergic sensitization is unknown because we have not yet developed a detailed understanding of the relationship between the level of mouse allergen exposure and the resulting mouse-specific immune responses.

A circumstance where the peak risk of allergic sensitization may occur at moderate, rather than high, levels of exposure is in the setting of domestic exposure to furred pet allergens. Platts-Mills and colleagues [7–9] have suggested that high levels of cat allergen exposure result

Supported by: NIAID AI07007 and AI 62974; RR 12552, NCRR, NIH; NIEHS ES09606, EPA R82674, NHLBI HL058942, and AAAAI President's Grant-In-Aid Award.

in a 'modified Th2 response' to cat allergen, characterized by high serum levels of cat-specific IgG and IgG4 and the absence of cat-specific IgE. If high level exposure induces a protective immune response, then reduction of exposure from high to moderate levels may actually confer an increased risk of allergic sensitization. It is important to determine whether this paradigm holds true for mouse allergen exposure before designing and implementing exposure reduction strategies.

We therefore examined the relationships between level of exposure, mouse-specific IgG and IgG4 levels, and risk of mouse skin test sensitivity among employees of a mouse research and production facility to determine if these non-IgE antibody responses are associated with exposure and IgE-mediated mouse sensitization. We hypothesized that higher mouse-specific IgG and IgG4 levels would be associated with an attenuated risk of mouse skin test sensitivity.

Methods

Study design and study population

A cross-sectional study of Jackson Laboratory employees was conducted to examine the relationships between mouse-specific IgG and IgG4 levels and risk of mouse skin test sensitivity. All employees of The Jackson Laboratory were eligible to participate in the study and participants provided written informed consent. The protocol was approved by the Institutional Review Boards at the Johns Hopkins University School of Medicine and The Jackson Laboratory. Two hundred and eighty-four of 1200 (24%) employees participated. Two hundred sixty participants were identified with valid skin test and mouse-specific IgG and IgG4 results.

Questionnaire

Sociodemographic, family, and medical histories were collected from participants using a self-administered questionnaire that was composed of questions from the validated American Thoracic Society respiratory symptom questionnaire [10] and the Collaborative Study on the Genetics of Asthma questionnaire [11].

Skin testing and mouse-specific immunoglobulin E

Skin tests to cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, ragweed, grass, oak, *Alternaria*, *Aspergillus*, and rat, mouse, hamster, guinea-pig, and rabbit epithelia were performed using full strength glycerinated extracts with the MultiTest[®] device (Lincoln Diagnostics, Decatur, IL, USA). A skin test was considered positive if the orthogonal weal diameter was at least 3 mm greater than the negative control and at least $\frac{1}{2}$ the size of

the histamine control. Serum levels of mouse allergen-specific IgE were quantified using the ImmunoCap 100 System (Pharmacia Diagnostics, Uppsala, Sweden). A mouse urine CAP-RAST value ≥ 0.35 kUA/L was considered positive.

Mouse-specific immunoglobulin G and immunoglobulin G4

Mouse-specific IgG was measured in serum samples using a solid phase antigen-binding assay as described previously [12]. Briefly, serum was incubated overnight with Protein G for determination of IgG1, IgG2, IgG3, and IgG4 (CNBr-activated Sepharose 4B; Pharmacia). Per test, 1–20 μ L serum was added to 500 μ g Sepharose in a total volume of 800 μ L (PBS/0.3% human serum albumin/0.1% Tween 20) together with radiolabelled mouse urinary protein 8 (MUP 8, a Mus m 1 isoform) for detection (kindly provided by Dr M. Stone) [13]. After washing, the amount of bound radioactivity was measured and read from a standard curve, a human pooled reference serum. Mouse-specific IgG4 levels were determined using anti-IgG4 solid phase (CNBr-activated Sepharose 4B; Pharmacia). Again, 1–20 μ L serum was added per test to 500 μ g Sepharose in a total volume of 800 μ L (PBS/0.3% human serum albumin/0.1% Tween 20). After overnight incubation, the samples were washed and incubated overnight with radiolabelled MUP 8 and the amount of bound radioactivity was measured. The previously mentioned reference serum was used as a standard. The results are expressed in arbitrary units per millilitre serum (AU/mL) and the detection limit of the antigen-binding assay was 0.5 AU/mL for IgG and 2.0 AU/mL for IgG4.

Exposure assessment

Airborne mouse allergen exposure measures were available for 154 of the participants. Air samples were collected throughout The Jackson Laboratory from January 2000 to April 2002 as described previously [3]. Each area was sampled from Monday through Thursday using a 20 L/min air sampling pump and an impactor (Air Diagnostics, Harrison, ME, USA). Protein was extracted from the filters using a standardized protocol and Mus m 1 was quantified by sandwich ELISA using immunosorbant purified sheep anti-Mus m 1 (kindly supplied by Dr J Ohman) [14]. Purified Mus m 1 was used for the calibration curve [15]. The 4 day average airborne Mus m 1 level was taken as the average room exposure. Study participants identified a primary area of work and the Mus m 1 level in that room was used as a measure of current exposure. In order to take duration of exposure into account, cumulative exposure was estimated as average Mus m 1 concentration multiplied by the duration of employment with resulting units of ng/m³ years. Participants were also asked if they

currently handled mice as an additional measure of exposure.

Statistical analyses

All analyses were performed with StataSE 8.0 (College Station, TX, USA). The distributions of dependent and independent variables were examined and their relationships to skin test sensitivity were examined using cross-tabulations and the χ^2 test or by displaying distributions of continuous variables by skin test status. Continuous variables were compared using the Mann–Whitney *U*-test or the Student's *t*-test, depending on the distribution of the variable. Spearman's correlation was used to analyse the relationships between exposure and mouse-specific IgG and IgG4 levels. Odds ratios (OR) and 95% confidence intervals (CIs) were generated using logistic regression. For regression analyses, mouse-specific IgG and IgG4 were $\log_{10}$ -transformed and levels below detection were assigned a value of $\frac{1}{2}$ of the limit of detection.

The relationships between mouse skin test sensitivity and mouse-specific IgG and IgG4 levels were explored using locally weighted regression (lowess function, bandwidth 0.8) to fit smoothed lines to the data to estimate the odds of mouse skin test sensitivity as a function of mouse-specific IgG or IgG4 levels. The observed trends suggested that the odds of skin test sensitivity peaked at moderate antibody levels, and decreased at the highest levels of both mouse-specific IgG and IgG4. Therefore, quadratic terms were included in the multivariate logistic regression models and these models were compared with models that

did not include quadratic terms using the likelihood ratio test. The models were tested for goodness of fit using the Hosmer–Lemeshow goodness-of-fit test. Atopy was defined as at least one positive skin prick test (SPT), aside from mouse. The threshold selected for statistical significance was $P < 0.05$ for all analyses.

Results

Of the 260 study participants, 60% were female, 59% handled mice, and 57% were animal caretakers or laboratory technicians (Table 1). The mean age was 38 years, and the median duration of employment at The Jackson Laboratory was 3 years. Over 18% of participants reported physician-diagnosed asthma, 20% had self-reported allergic rhinitis (AR), and 50% had at least one positive skin test. Almost 19% of participants had a positive SPT to mouse, and 10% had detectable mouse-specific IgE. Female gender, mouse handling, physician-diagnosed asthma, and self-reported AR were all significantly associated with mouse skin test sensitivity. There was no difference in age between the skin test positive and skin test negative groups, but the skin test positive group had been employed at The Jackson Laboratory longer, on average, than the skin test negative group (4 vs. 2 years, $P = 0.01$).

Two hundred and nineteen (84%) participants had detectable mouse-specific IgG, and 72 (28%) had detectable mouse-specific IgG4. Mouse-specific IgG and IgG4 levels were strongly correlated ($r_s = 0.67$, $P < 0.0001$, Fig. 1.) and mouse-specific IgG4 and IgG were both positively correlated with cumulative exposure ($r_s = 0.31$,

Table 1. Skin test sensitivity and study population characteristics

Characteristic	Total population (260)	+ mSPT (49)	– mSPT (211)
Age (years), mean $\pm$ SD	38.0 $\pm$ 11.3	37.0 $\pm$ 12.0	38.2 $\pm$ 11.1
Sex*, n (%)			
Female	157 (60.4)	36 (73.5)	121 (57.3)
Male	103 (39.6)	13 (26.5)	90 (42.7)
Job category†, n (%)			
Administrative/support	81 (31.2)	7 (14.3)	74 (35.1)
Scientist	13 (5.0)	3 (6.1)	10 (4.7)
Laboratory technician	72 (27.7)	23 (46.9)	49 (23.2)
Animal caretaker	75 (28.9)	14 (28.6)	61 (28.9)
Other	19 (7.3)	2 (4.1)	17 (8.1)
Currently handle mice†, n (%)	152 (58.7)	38 (77.6)	114 (54.3)
Duration of employment† (years), median (IQR)	3 (1–7)	4 (2–14)	2 (1–7)
Airways disease, n (%)			
Asthma†	47 (18.2)	17 (34.7)	30 (14.3)
Self-reported allergic rhinitis*	52 (20.2)	16 (32.7)	36 (17.3)
Atopy†, n (%)	136 (52.3)	46 (93.9)	90 (42.7)
Cumulative exposure† (ng/m ³ years)	0.34 (0.05–1.28)	1.0 (0.27–3.28)	0.28 (0.04–0.91)

* $P < 0.05$, χ^2 test.

† $P \leq 0.01$, Mann–Whitney *U*-test or χ^2 test.

SPT, skin prick test.

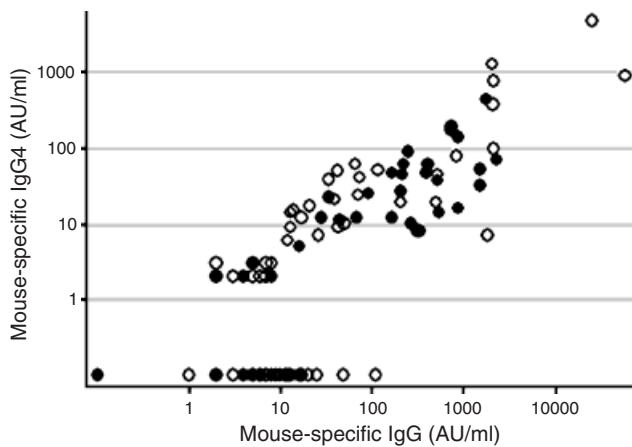


Fig. 1. Scatterplot of mouse-specific IgG vs. IgG4. IgG and IgG4 are plotted in arbitrary units per millilitre serum (AU/mL) on the x axis and y axis, respectively. ○ represent skin test negative participants, ● represent skin test positive participants. ($r_s = 0.67$, $P < 0.0001$)

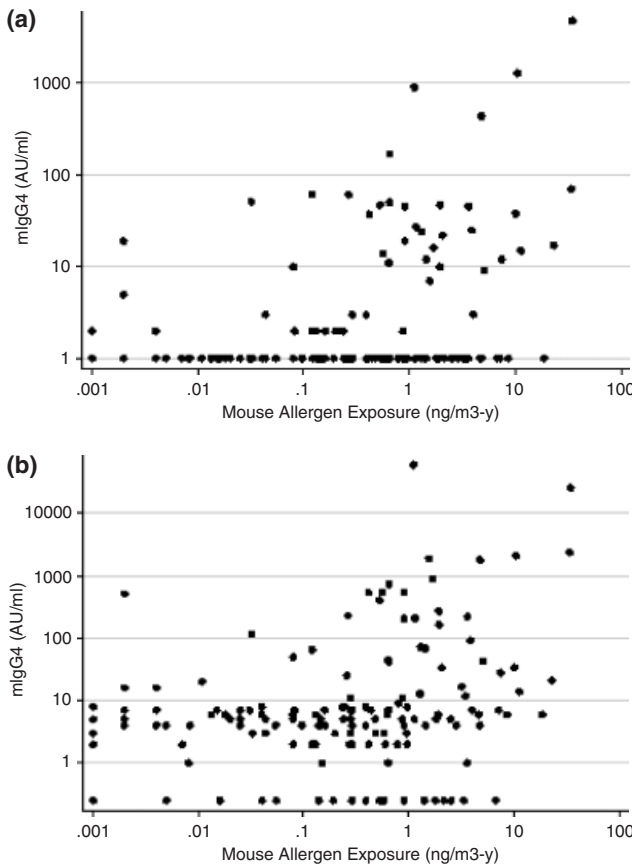


Fig. 2. Mouse-specific antibody levels and exposure. Mouse-specific IgG4 (a) and IgG (b) are plotted on the y axis, and mouse allergen exposure (ng/m³ years) is plotted on the x axis. $r_s = 0.31$, $P = 0.0001$ (IgG4 vs. exposure) and $r_s = 0.27$, $P = 0.0006$ (IgG vs. exposure).

$P = 0.0001$, and $r_s = 0.27$, $P = 0.0006$, respectively, Fig. 2). In bivariate analyses, mouse-specific IgG and IgG4 levels were higher in the skin test positive than the skin test

Table 2. Mouse-specific IgG and IgG4 levels

Mouse-specific antibody	+ mSPT (49)	– mSPT (211)	P-value
IgG, median (IQR)	34 (5–334)	6 (4–9)	< 0.0001
IgG4	10 (BD–37)	BD(BD–BD)	< 0.0001

BD, below detection; SPT, skin prick test.

Table 3. Mouse-specific IgG4 Levels and mouse skin test sensitivity

Variable	Model 1*†		Model 2*†		Model 3		Model 4	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
$\log_{10}(\text{mIgG4})$	2.6	1.7–3.8	21.1	6.2–71.6	9.5	2.4–37.7	6.2	1.0–38.6
$[\log_{10}(\text{mIgG4})]^2$	–	–	0.4	0.2–0.7	0.4	0.2–0.7	0.4	0.2–0.8
$\log_{10}(\text{mIgG})$	–	–	–	–	1.7	1.0–2.8	2.3	1.0–5.1

Models 1, 2, and 3 include only the variables listed in the table. Model 4 is also adjusted for atopy, sex, and mouse allergen exposure.

*Model 1 vs. 2, likelihood ratio test, $P = 0.0001$.

†Hosmer–Lemeshow goodness-of-fit tests: Model 1: $P = 0.03$; Model 2: $P = 0.95$.

OR, odds ratios; CI, confidence intervals.

negative group (Table 2). In addition, having a detectable mouse-specific IgG4 level was strongly associated with mouse skin test sensitivity (OR 6.4, 95% CI 3.3–12.4).

An analysis of the relationship between the continuous measure of mouse-specific IgG4 and skin test sensitivity indicated that the odds of skin test sensitivity did not increase monotonically with mouse-specific IgG4 levels. Instead, the odds decreased at higher mouse-specific IgG4 levels. Specifically, a logistic regression model that included a quadratic term for IgG4 (Table 3) provided a better fit of the data than a model that only included IgG4 (likelihood ratio test, $P = 0.0001$). The predicted line is an 'upside-down U' shape (Fig. 3), indicating that the odds of skin test sensitivity was decreasing at the highest levels of mouse-specific IgG4, although the 95% CI widened at the highest IgG4 levels because of fewer datapoints at higher than at lower IgG4 levels. The relationship between the mouse-specific IgG4 level and mouse skin test sensitivity did not change after adjusting for mouse-specific IgG, female sex, atopy, and cumulative exposure (Table 3).

On the other hand, the odds of skin test sensitivity did not appear to decrease at higher levels of mouse-specific IgG (Table 4). Instead, the odds increased monotonically with mouse-specific IgG levels. Specifically, a model including mouse-specific IgG alone demonstrated a positive association between mouse-specific IgG and skin test sensitivity (OR 2.1, 95% CI 1.5–2.8), and the addition of a quadratic term for mouse-specific IgG did not improve the overall fit of the model (likelihood ratio test, $P = 0.62$). The highest levels of mouse-specific IgG4, but not IgG, appear

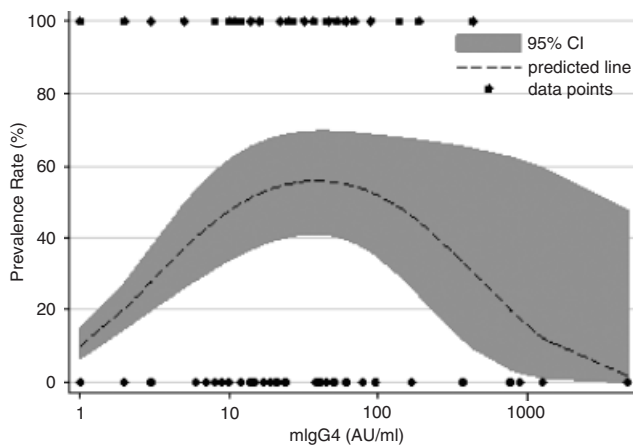


Fig. 3. Predicted prevalence rate of mouse skin test sensitivity as a function of mouse-specific IgG4. The prevalence rate of a +mouse skin prick test is represented on the y-axis and is plotted as a function of the mouse-specific IgG4 level as predicted from a logistic regression model (solid line). The 95% CI is represented by the shaded area, and the original data points are represented as •.

Table 4. Mouse-specific IgG levels and mouse skin test sensitivity

Variable	Model 5*		Model 6*	
	OR	95% CI	OR	95% CI
$\log_{10}(\text{mIgG})$	2.1	1.5–2.8	2.3	1.3–4.1
$[\log_{10}(\text{mIgG})]^2$	—	—	1.0	0.8–1.1

Models 5 and 6 include only the variables listed in the table.

*Model 5 vs. 6, likelihood ratio test, $P = 0.62$.

OR, odds ratios; CI, confidence intervals.

to be associated with a decreasing risk of skin test sensitivity.

Discussion

Our findings suggest that mouse-specific IgG4 is associated with an increased risk of mouse skin test sensitivity, but that very high levels of mouse-specific IgG4 may be associated with protection against skin test sensitivity. However, because there were relatively few datapoints at the highest IgG4 levels, a plateau or rise in the risk of skin test sensitivity at higher mouse-specific IgG4 levels cannot be definitively excluded. In addition, increasing cumulative mouse allergen exposure was associated with an increase in mouse-specific IgG4 levels. These findings suggest that small reductions in allergen exposure, from 'high' to 'moderate' levels, may have little impact on the risk of allergic sensitization, and that primary prevention of allergic sensitization may best be achieved by reducing exposure to the lowest levels.

Consistent with our findings, Portengen et al. [16] found that mouse-specific IgG4 increased with increasing

exposure, and IgG4 levels were higher among IgE sensitized workers than non-sensitized workers. We also found that mouse-specific IgG4 levels and cumulative mouse allergen exposure were positively correlated, and that having a detectable level of mouse-specific IgG4 conferred more than a sixfold increase in the odds of skin test sensitivity. If the mouse-specific IgG4 response occurs before the development of the IgE response, then mouse-specific IgG4 levels may provide a means of identifying workers who are at highest risk of developing skin test sensitivity, so that preventive measures can be appropriately targeted.

In contrast to the study by Portengen et al., our worker population with the highest specific IgG4 levels had an attenuated risk of skin test sensitivity. Although our results may be due to random chance because of the small number of study participants with very high mouse allergen-specific IgG4 levels, the findings suggest that the probability of skin test sensitivity may decrease at the highest mouse allergen-specific IgG4 levels. It is also possible that the sparse data at the highest levels of mouse allergen-specific IgG4 unduly influenced the fit of the logistic regression model, although goodness-of-fit testing suggests that the final model provides a good representation of the actual data. Ultimately, though, the relatively sparse data at the highest IgG4 levels do not allow any definitive conclusions to be drawn about the relationship between high mouse allergen-specific IgG4 levels and skin test sensitivity. In addition, the attenuated risk of skin test sensitivity was seen only at the very highest IgG4 levels, so that mouse-specific IgG4 may be a marker of protection against allergic sensitization, but only in a small minority of workers. Larger, prospective studies are needed to provide a more precise estimate of the risk of skin test sensitivity at the highest IgG4 levels.

There are also several differences between our study and the Portengen study that could account for these apparent discrepancies. First, although rat and mouse allergens share some characteristics, they are not the same allergen and therefore may elicit different immune responses. Second, the methods used for measuring allergen-specific IgG4 levels differed in the two studies. In our study, a solid-phase antigen-binding assay with the major allergen as the substrate was used. In the Portengen study, an enzyme immunoassay was used to quantify allergen-specific IgG4, and the substrate was crude extract rather than purified allergen. More prospective studies are needed to determine if the difference between the studies can be explained by differences in the two allergens or in the assay techniques.

Although workers with the highest levels of exposure also tended to have the highest mouse-specific IgG4 levels, there were some highly exposed workers who did not have particularly high mouse-specific IgG4 levels. Two possible explanations include exposure

misclassification and modification of the exposure-response relationship by genetic differences within the study population. Exposure misclassification occurs to some extent in every study of allergen exposure. The exposure metric used to estimate cumulative exposure in this study, while an improvement over a one-time measure of exposure intensity, may still misclassify some participants so that there may be some workers classified as highly exposed whose exposure was actually lower.

In addition, workers with particular genetic polymorphisms may be more or less susceptible to mounting an IgG4 response, so that only participants with a particular genetic make-up would manifest enhanced production of specific IgG4 in response to high exposure. For example, Reefer *et al.* [17] found that a particular HLA-DRB1 allele, *0701, was associated with both a cat allergen-specific IL-10 response and a cat-specific IgG4 response. These findings suggest a gene-environment interaction and may explain why the phenomenon of high-dose protection against allergic sensitization has not been found consistently across studies: simply put, the genotypic make-up of a study population may be a significant determinant of whether high-dose protection is observed in any given epidemiologic study. It is not surprising, then, that we have not observed a direct relationship between high levels of cumulative exposure and decreased risk of allergic sensitization [18] as some highly exposed workers did not mount a robust IgG4 response. Genetic studies are needed to determine if particular HLA alleles are associated with protection against developing allergic sensitization to mouse allergen.

Although these findings suggest that prolonged, high dose exposure to mouse allergen can result in an enhanced IgG4 response to mouse allergen, the limitations associated with a cross-sectional study should be taken into consideration. For example, the temporal relationships of exposure, mouse-specific IgG responses, and mouse skin test sensitivity cannot be ascertained in a cross-sectional study, so that the observed associations could be due to the fact that non-IgE-sensitized workers may have greater cumulative exposure levels than IgE-sensitized workers because they are more likely to be asymptomatic when exposed to mouse allergen. The higher levels of cumulative exposure, in turn, could drive mouse-specific IgG4 responses. However, our results indicate that IgE-sensitized workers had higher levels of cumulative exposure than non-IgE-sensitized workers, suggesting that healthy worker bias had little impact on the findings. Ultimately, though, healthy worker bias can only be excluded by conducting a prospective study in which workers who leave their jobs entirely are tracked, and changes in allergen exposure are carefully assessed to account for any alterations in a worker's duties.

Our findings suggest that mouse-specific IgG4 may be a risk factor for the development of IgE-sensitization to

mouse allergen. In addition, workers with the most pronounced IgG4 responses may be at decreased risk of mouse skin test sensitivity. Prospective studies should be conducted to determine if a pronounced IgG4 response is associated with protection against skin test sensitivity.

References

- 1 Phipatanakul W, Eggleston PA, Wright EC, Wood RA. Mouse allergen. I. The prevalence of mouse allergen in inner-city homes. The National Cooperative Inner-City Asthma Study. *J Allergy Clin Immunol* 2000; **106**:1070-4.
- 2 Phipatanakul W, Eggleston PA, Wright EC, Wood RA. Mouse allergen. II. The relationship of mouse allergen exposure to mouse sensitization and asthma morbidity in inner-city children with asthma. *J Allergy Clin Immunol* 2000; **106**:1075-80.
- 3 Matsui EC, Krop EJ, Diette GB, Aalberse RC, Smith AL, Eggleston PA. Mouse allergen exposure and immunologic responses: IgE-mediated mouse sensitization, mouse-specific IgG and IgG4 levels. *Ann Allergy Asthma Immunol* 2004; **93**:171-8.
- 4 Reeb C, Jones R, Bearg D, Bedigan H, Myers D, Paigen B. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. *Contemp Top Lab Anim Sci* 1998; **37**:43-9.
- 5 Reeb CK, Jones RB, Bearg DW, Bedigian H, Paigen B. Impact of room ventilation rates on mouse cage ventilation and micro-environment. *Contemp Top Lab Anim Sci* 1997; **36**:74-9.
- 6 Reeb-Whitaker CK, Harrison DJ, Jones RB, Kacergis JB, Myers DD, Paigen B. Control strategies for aeroallergens in an animal facility. *J Allergy Clin Immunol* 1999; **103**:139-46.
- 7 Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; **357**:752-6.
- 8 Hesselmar B, Aberg B, Eriksson B, Bjorksten B, Aberg N. High-dose exposure to cat is associated with clinical tolerance—a modified Th2 immune response? *Clin Exp Allergy* 2003; **33**: 1681-5.
- 9 Platts-Mills TA, Woodfolk JA, Erwin EA, Aalberse R. Mechanisms of tolerance to inhalant allergens: the relevance of a modified Th2 response to allergens from domestic animals. *Springer Semin Immunopathol* 2004; **25**:271-9.
- 10 Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978; **118**:1-120.
- 11 Barnes KC, Freidhoff LR, Horowitz EM *et al.* Physician-derived asthma diagnoses made on the basis of questionnaire data are in good agreement with interview-based diagnoses and are not affected by objective tests. *J Allergy Clin Immunol* 1999; **104**: 791-6.
- 12 Witterman AM, Stapel SO, Sjamsoedin DH, Jansen HM, Aalberse RC, Van Der Zee JS. Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int Arch Allergy Immunol* 1996; **109**:369-75.
- 13 Sharrow SD, Novotny MV, Stone MJ. Thermodynamic analysis of binding between mouse major urinary protein-I and the pheromone 2-sec-butyl-4,5-dihydrothiazole. *Biochemistry* 2003; **42**:6302-9.
- 14 Ohman JL Jr, Hagberg K, MacDonald MR, Jones RR Jr, Paigen BJ, Kacergis JB. Distribution of airborne mouse allergen in a

- major mouse breeding facility. *J Allergy Clin Immunol* 1994; **94**:810–7.
- 15 Chen P, Eggleston PA. Allergenic proteins are fragmented in low concentrations of sodium hypochlorite. *Clin Exp Allergy* 2001; **31**:1086–93.
- 16 Portengen L, de Meer G, Doekes G, Heederik D. Immunoglobulin G4 antibodies to rat urinary allergens, sensitization and symptomatic allergy in laboratory animal workers. *Clin Exp Allergy* 2004; **34**:1243–50.
- 17 Reefer AJ, Carneiro RM, Custis NJ *et al.* A role for IL-10-mediated HLA-DR7-restricted T cell-dependent events in development of the modified Th2 response to cat allergen. *J Immunol* 2004; **172**:2763–72.
- 18 Matsui EC, Krop EJ, Diette GB, Aalberse RC, Smith AL, Eggleston PA. Mouse allergen exposure and immunologic responses: IgE-mediated mouse sensitization and mouse specific IgG and IgG4 levels. *Ann Allergy Asthma Immunol* 2004; **93**: 171–8.