

Mouse allergen-specific immunoglobulin G and immunoglobulin G4 and allergic symptoms in immunoglobulin E-sensitized laboratory animal workers

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Summary

Background High levels of allergen-specific IgG have been associated with clinical efficacy in immunotherapy studies, but whether this antibody isotype is associated with clinical tolerance in the setting of environmental exposure remains unclear.

Objective To determine if mouse allergen-specific IgG (mIgG) and IgG4 (mIgG4) levels are associated with mouse-related symptoms among IgE-sensitized laboratory workers.

Methods Fifty-eight workers with either skin test or serologic evidence of IgE-mediated mouse sensitization were studied. Symptom data were obtained by a questionnaire. Serum levels of mouse-specific IgG, IgG4, and IgE were quantified by a solid-phase antigen-binding assay (IgG) and RAST (IgG4 and IgE), and the relationships between mouse-specific serologic responses and mouse-related symptoms were analysed.

Results Twenty-three (39.7%) participants reported mouse-related symptoms. Mouse-specific IgG and IgG4 levels were not associated with mouse-related symptoms among the study population as a whole. Among the 29 (50%) participants with detectable mouse-specific IgE (mIgE), higher mouse-specific IgG and IgG4 levels were associated with a decreased risk of symptoms, after adjusting for mIgE level (odds ratio (OR) 0.3, 95% confidence interval (CI): 0.1–1.4, and OR 0.3, 95% CI: 0.04–2.6, respectively). Higher levels of mIgG and mIgG4 remained associated with a decreased risk of symptoms after additional adjustment for sex and handling of mice (OR 0.1, 95% CI: 0.02–0.7, and OR 0.2, 95% CI: 0.02–2.1, respectively). Higher mIgG:IgE and mIgG4:IgE ratios were also associated with a decreased risk of symptoms after adjusting for these confounders (OR 0.1, 95% CI: 0.02–0.7, and OR 0.2, 95% CI: 0.02–0.92, respectively).

Conclusion Among workers with detectable mIgE, higher mIgG and mIgG4 levels are associated with a decreased risk of mouse-related symptoms. High serum levels of mIgG or mIgG4 may be markers for clinical tolerance among laboratory mouse workers with detectable mIgE, but these findings need to be confirmed in larger, prospective studies.

Keywords allergen-specific IgG, IgG4, laboratory animal allergy, mouse allergen

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Introduction

Mouse allergy affects as many as one-third of laboratory animal workers [1–3] and has recently been identified as an important problem among children with asthma [4–6]. Although skin test sensitivity and serologic evidence of IgE antibody to mouse allergen are risk factors for having mouse-related allergy symptoms, only about half of the individuals with positive diagnostic tests for specific IgE are symptomatic [1, 2, 7]. A better understanding of the factors that distinguish asymptomatic from symptomatic IgE-sensitized individuals could lend insight into the pathogenesis of clinical tolerance and help in the identification of IgE-sensitized individuals

who are at highest risk of developing mouse-related symptoms.

Factors such as weal size, specific IgE level, and degree of exposure have been associated with the symptomatic status of IgE-sensitized individuals and should therefore be considered when examining predictors of symptomatic status [2, 8–10]. For example, higher specific IgE levels or larger skin prick test (SPT) weal sizes may confer an increased risk of having chronic allergic symptoms [1, 2]. In addition, IgE-sensitized individuals with higher levels of exposure may be more symptomatic [10, 11]. Because exposure is associated with both symptoms and allergen-specific immunologic markers, it is an important confounder of these relationships. Clinical tolerance, then, may be best defined as the lack of symptoms in the setting of both IgE-mediated sensitization and exposure.

Other immunologic markers, such as specific IgG and IgG4, have been associated with a decreased risk of

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developing IgE-mediated sensitization in some settings [12], but these antibody responses may also modify the symptomatic status of IgE-sensitized individuals, leading to clinical tolerance. During immunotherapy, for example, prolonged, regular doses of allergen result in a marked increase in allergen-specific IgG and IgG4 at the same time that symptoms improve [13–15]. Furthermore, these changes in allergen-specific IgG and IgG4 and symptoms are accompanied by only a modest decrease in allergen-specific IgE. In fact, some studies have reported an association between allergen-specific IgG or IgG4 levels and clinical efficacy of immunotherapy [14–17]. Several mechanisms whereby specific IgG may protect against symptoms have been proposed. For example, allergen-specific IgG has been shown to inhibit basophil histamine release [18] and IgE-facilitated antigen presentation by blocking formation of allergen–IgE complexes [19, 20]. In addition, IgG that is specific for Fel d 1 has been shown to increase the threshold dose for eliciting a positive skin test to cat [21]. These mechanistic findings suggest that the observed association between allergen-specific IgG levels and clinical tolerance is biologically plausible.

The association of specific IgG antibody levels with clinical tolerance has not been well studied in the setting of environmental exposure. For mouse allergen, we have previously reported that high levels of specific IgG and IgG4 are associated with IgE-mediated mouse sensitization among laboratory animal workers [7], but it is unclear if these antibody responses modify the symptomatic status of IgE-sensitized individuals. A marker of clinical tolerance, such as specific IgG or IgG4, could lend insight into potential treatment strategies as well as help in the identification of individuals at greatest risk of developing symptoms. A serologic biomarker of risk could be helpful in determining which workers would be likely to benefit from preventive measures. Therefore, to determine if mouse-specific IgG levels are associated with clinical tolerance, we examined the relationships between mouse-specific IgG and IgG4 and mouse-related symptoms among a group of IgE-sensitized workers at a mouse research and production facility.

Methods

Study design and study population

A cross-sectional study of adults with IgE-mediated mouse sensitization was conducted to compare mouse allergen-specific IgE, IgG and IgG4, and mouse SPT weal size between workers with and without mouse allergy symptoms. 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 School of Medicine and The Jackson Laboratory. Two hundred and eighty-four out of 1200 (24%) employees participated. One hundred and fifty-one of these participants had available exposure measures and the relationships between exposure and mouse-specific antibody levels and skin test sensitivity have been described elsewhere [7]. Fifty-eight participants were identified, who had either a positive mouse SPT, detectable serum mouse-specific IgE, or both.

Questionnaire

Participants completed a self-administered questionnaire that was composed of the validated ATS respiratory symptom questionnaire [22] and a questionnaire adapted from the Collaborative Study on the Genetics of Asthma questionnaire [23]. A participant was considered to have had ocular symptoms with exposure to mice if he/she responded positively to 'Have you been bothered by itchy, watery eyes IN THE PAST 12 MONTHS as a result of being near lab mice?' Similarly, the following phrases were used to assess other mouse-related symptoms: 'problem with sneezing or runny nose?', 'welts or hives?', 'cough?', 'chest sounded wheezy or whistling?', and 'bothered by shortness of breath or tightness in your chest as a result of being near lab mice?' A participant was considered symptomatic if he/she reported mouse-related nasal or ocular symptoms in the past 12 months. This definition was chosen because there were only two participants reporting dermatologic or lower respiratory symptoms who did not also report nasal or ocular symptoms.

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 1/2 the size of the histamine control. Serum levels of mouse allergen-specific IgE were quantified using the ImmunoCap 100 System and mouse urine immunocaps (Pharmacia Diagnostics, Uppsala, Sweden). A mouse urine CAP-RAST value ≥ 0.35 kU_A/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 previously described [21]. Briefly, serum was incubated overnight with Protein G for determination of IgG1, IgG2, IgG3, and IgG4 (CNBr-activated Sepharose 4B; Pharmacia Diagnostics). 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, Merk-Schuchardt, Hobenbrun, Germany) together with radiolabelled mouse urinary protein 8 (MUP 8, a Mus m 1 isoform) for detection (kindly provided by Dr M. Stone) [24]. After washing, the amount of bound radioactivity was measured and read from a standard curve, a human pooled reference serum. The results are expressed in arbitrary units per millilitre serum (AU/mL) and the detection limit of the antigen-binding assay for IgG was 0.5 AU/mL. Mouse-specific IgG4 was determined by RAST. Dried mouse urine was dissolved in PBS and coupled onto a solid phase (CNBr-activated Sepharose 4B; Pharmacia Diagnostics, 100 μ g protein to 100 mg Sepharose). Per test, 5–40 μ L serum was added to 500 μ g Sepharose in a total volume of 300 μ L (PBS/0.3% human serum albumin/0.1% Tween 20). For the detection of IgG4 antibodies, ¹²⁵I-labelled anti-human IgG4

was used. All tests were done in duplicate and averaged. Results were expressed as percentage binding of radioactivity, corrected for background values. The amount of radioactivity was measured and read from a standard dilution curve obtained with a reference serum and Der p 2 Sepharose. Results were expressed in arbitrary units per millilitre.

Exposure assessment

Airborne mouse allergen exposure measures were available for 38 of the participants. Air samples were collected throughout The Jackson Laboratory from January 2000 to April 2002. Each area was sampled from Monday to Thursday, 08:00–16:00 hours, using a 20 L/min air sampling pump and an impactor (Air Diagnostics, Harrison, ME, USA), and a new filter was used for each 8 h shift. 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) [25]. Purified Mus m 1 was used for the calibration curve [26]. 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. The limit of detection was 0.33 ng/air filter, which is equivalent to 0.03 ng/m³ for a typical 8 h sample.

Statistical analyses

All analyses were performed using Stata SE 8.0 (Stata Corp, College Station, TX, USA). The distributions of dependent and independent variables were examined and their relationships to symptomatic status were examined using cross-tabulations and the χ^2 test or by displaying distributions of continuous variables by symptomatic status. Continuous variables were compared using the Mann–Whitney *U*-test or the Student *t*-test, depending on the distribution of the variable. Spearman's rank correlation was used to examine relationships between continuous variables. For mouse-specific IgG, IgG4, and IgE, levels below detection were assigned a value 1/2 of the limit of detection. Because of the skewed distributions of mouse-specific IgG, IgG4, and IgE, these variables were log-transformed when analysed as continuous variables in regression models.

The ratio of mIgG4 to mIgE (mIgG4:IgE) was calculated as a measure of the relative level of mouse-specific, IL-10-driven antibody levels to mIgE levels. This ratio is based on the conceptual model that immune responses characterized primarily by Th2 cytokines lead to production of both IgG4 and IgE, while immune responses characterized primarily by IL-10 lead to enhanced production of IgG4, resulting in higher IgG4 levels, relative to IgE levels. Other investigators have applied this conceptual framework in a study of allergen immunotherapy and found that the allergen-specific IgG4:IgE ratio was a marker of clinical tolerance [15]. We also analysed the ratio of mIgG to mIgE (mIgG:IgE) to

determine if any effect associated with the mIgG4:IgE level was specific to the IgG4 subclass, or if the effect was also seen with a measure of all four IgG subclasses. The ratios were log₁₀ transformed and the relationships between these ratios and risk of symptoms were examined using both non-parametric modelling (lowess function, bandwidth 0.8) and logistic regression.

The subgroup of participants with detectable mIgE was examined to determine if the effect of mouse-specific IgG and IgG4 on symptoms differed among participants with both positive SPTs and detectable mIgE. This subgroup was selected for several reasons. First, this mIgE positive group was likely to differ from the mIgE-negative group by either degree of IgE sensitization or by the specific mouse proteins to which they were sensitized. Second, calculating a mIgG:IgE or mIgG4:IgE ratio for the group with undetectable mIgE requires assigning an arbitrary value to a mIgE < 0.35 kU_A/L, and this potential source of bias could be avoided by restricting the analyses to participants with measurable mIgE. Third, preliminary analyses suggested that the protective effect of mIgG and mIgG4 was primarily seen among participants with mIgE levels > 0.35 kU_A/L.

Simple logistic regression was used to generate crude odds ratios (ORs) and 95% confidence intervals (CIs), and multiple logistic regression was used to adjust for potential confounders. Sex of the participant was an important confounder and was included as a covariate in the final analyses. Even though there were no significant differences in measures of current and cumulative exposure between symptomatic and asymptomatic participants, current handling of mice was associated with symptoms and mouse-specific antibody levels. Therefore, the final analyses were adjusted for exposure by including a covariate indicating if the participant was a mouse handler.

Two study participants who were receiving mouse immunotherapy were excluded from the analyses that included mouse-specific IgG and IgG4. The threshold selected for statistical significance was *P* < 0.05 for all analyses.

Results

Study population

Fifty-eight employees of The Jackson Laboratory with either a positive mouse SPT or detectable mouse-specific IgE antibody, or both, participated. Twenty-nine of the participants (50%) had a detectable level of mIgE. The median mIgE was 0.39 kU_A/L and the median mouse SPT weal size was 6.5 mm (Table 1). The mean age was 36.9 years, 69.0% of participants were female, and the median duration of employment was 4 years. Physician-diagnosed asthma was reported by 36.2%, and 32.8% of participants reported allergic rhinitis. The study population included administrative personnel and scientists; however, animal caretakers and laboratory technicians made up over 75% of the study population. Forty-three (74.1%) of IgE-sensitized employees currently handled mice.

Twenty-three (39.7%) of the participants reported having mouse-related nasal or ocular symptoms (Table 2). There was a higher proportion of mouse handlers in the symptomatic

Table 1. Study population characteristics

Characteristic	Study population (58)
Age (years), mean $\pm$ SD	36.9 $\pm$ 11.5
Sex, <i>n</i> (%)	
Male	18 (31.0)
Female	40 (69.0)
Job category, <i>n</i> (%)	
Administrative/support	8 (13.8)
Scientist	4 (6.9)
Laboratory technician	27 (46.6)
Animal caretaker	17 (29.3)
Other	2 (3.4)
Currently handle mice, <i>n</i> (%)	43 (74.1)
Duration of employment (years), median (IQR)	4 (2–14)
Airways disease, <i>n</i> (%)	
Asthma	21 (36.2)
Self-reported allergic rhinitis	19 (32.8)
Mouse-related symptoms, previous 12 months	
Ocular and/or nasal	23 (39.7)
Lower respiratory	11 (19.0)
Dermatologic	5 (8.8)
Measures of mouse-specific IgE	
mIgE (kU _A /L), median (IQR)*	0.39 (<0.35–1.41)
SPT weal size (mm), median (IQR)**	6.5 (4.5–8.0)

*Two missing. **Three missing.
SPT, skin prick test.

Table 2. Sociodemographic and worker characteristics and mouse-related symptoms

Characteristic	Symptomatic (<i>n</i> = 23)	Asymptomatic (<i>n</i> = 35)
Age (years), mean $\pm$ SD	34.5 $\pm$ 10.7	38.6 $\pm$ 11.9
Female, <i>n</i> (%)	18 (78.3)	22 (62.9)
Job category, <i>n</i> (%)		
Administrative/support	1 (4.4)	7 (20.0)
Scientist	0 (0)	4 (11.4)
Laboratory technician*	15 (65.2)	12 (34.3)
Animal caretaker	6 (26.0)	11 (31.4)
Other	1 (4.4)	1 (2.9)
Currently handle mice, <i>n</i> (%)*	21 (91.3)	22 (62.9)
Airways disease, <i>n</i> (%)		
Asthma	9 (39.1)	12 (34.3)
Self-reported allergic rhinitis	10 (43.5)	9 (25.7)

**P* = 0.02.

group as compared with the asymptomatic group (91.3% vs. 62.9%, *P* = 0.02). Laboratory technicians also made up a higher proportion of the symptomatic group than the asymptomatic group (65.2% vs. 34.3%, *P* = 0.02). There were no statistically significant differences between the two groups with respect to age, sex, other job categories, and history of allergic disease.

Immunologic and exposure characteristics and mouse-related symptoms

Serum levels of mIgG and mIgG4 were higher, in general, among symptomatic participants than asymptomatic partici-

pants, but the differences were not statistically significant (Table 3). Measures of IgE-mediated sensitization were statistically significantly higher in the symptomatic group than the asymptomatic group (mIgE: 0.67 vs. <0.35 kU_A/L, respectively; *P* = 0.005, and weal size: 7.3 vs. 5.5, respectively; *P* = 0.02). Mouse-specific IgE levels were correlated with mIgG and mIgG4 levels (r_s = 0.60, *P* < 0.0001 and r_s = 0.40, *P* = 0.003, respectively). Cumulative exposure and mIgG and mIgG4 were also correlated (r_s = 0.32, *P* = 0.06 and r_s = 0.36, *P* = 0.03, respectively).

There were no statistically significant differences in mIgG:IgE and mIgG4:IgE between the asymptomatic and symptomatic groups, but there was a trend towards higher mIgG4:IgE in the asymptomatic group (6.5 vs. 3.2, *P* = 0.10). Duration of employment and mouse allergen exposure were not significantly different between the two groups.

Mouse-specific immunoglobulin G and immunoglobulin G4 and risk of symptomatic mouse allergy

Among the study population as a whole, higher mIgG, mIgG4 and mIgG:IgE ratios were not associated with risk of mouse-related symptoms (Table 4). However, higher mIgG4:IgE ratios were associated with a decreased risk of having allergic symptoms (OR 0.4, 95% CI: 0.2–1.0).

For participants with mIgE $\geq$ 0.35 kU_A/L, higher mouse-specific IgG and IgG4 levels were associated with a decreased risk of mouse-related symptoms (Table 5). After adjusting for sex and current mouse handling, every log₁₀ increase in mIgG level was associated with a decreased risk of mouse-related symptoms (OR 0.1; 95% CI: 0.02–0.7). Similarly, every log₁₀ increase in the mIgG4 level was associated with a decreased risk of symptoms, but this association was not statistically significant (OR 0.2; 95% CI: 0.02–2.1).

Higher ratios of mIgG:IgE and mIgG4:IgE were also associated with a decreased risk of symptoms among participants with mIgE $\geq$ 0.35 kU_A/L (Table 4 and Fig. 1). After adjusting for sex and handling of mice, higher mIgG:IgE and mIgG4:IgE ratios were both independently associated with a decreased risk of symptoms (OR 0.2; 95% CI: 0.02–0.9 and OR 0.1; 95% CI: 0.02–0.7, respectively).

The relationships between mIgG and mIgG4 and risk of symptoms were similar when the analyses were restricted to mouse handlers only. Among mouse handlers with detectable mIgE, mIgG and mIgG4 were associated with a decreased risk of symptoms, after adjusting for mIgE and sex (OR 0.2; 95% CI: 0.03–1.0, and OR 0.3; 95% CI: 0.03–3.0, respectively). Similarly, the mIgG:IgE and mIgG4:IgE ratios were also associated with a decreased risk of symptoms after adjusting for sex (OR 0.2; 95% CI: 0.03–0.8 and OR 0.2; 95% CI: 0.03–1.1, respectively).

Discussion

This study provides evidence that high levels of mouse-specific IgG and IgG4 are associated with a decreased risk of symptomatic mouse allergy. These findings, taken together with previous work indicating that allergen-specific IgG and IgG4 increase with increasing exposure [2, 7, 27, 28], suggest

Table 3. Exposure and immunologic variables and mouse-related symptoms

	Symptomatic (<i>n</i> = 23)	Asymptomatic (<i>n</i> = 35)	<i>P</i>
Mouse-IgG (AU/mL)*	164 (36.5–434)	8 (5–402)	0.08
Mouse- IgG4 (AU/mL)*	12 (5.5–39)	2 (BD–28)	0.13
mIgE (kU _A /L)†	0.67 (<0.35–2.78)	<0.35 (<0.35–0.52)	0.005
Weal size (mm)†	7.25 (6.5–8.5)	5.5 (4.5–7.0)	0.02
mIgG : mIgE*	61.95 (22.9–238.7)	41.2 (23.5–385.0)	0.94
m IgG4 : mIgE*	3.2 (1.1–11.6)	6.5 (3.5–16.5)	0.10
Employment duration (years)	4 (2–14)	3 (2–14)	0.99
Cumulative exposure (ng/m ³ years)§	0.78 (0.52–1.95)	0.87 (0.15–3.36)	0.86
Current exposure (ng/m ³)§	0.14 (0.13–0.29)	0.29 (0.13–0.53)	0.55

Results are presented as median (IQR).

*Two participants receiving immunotherapy excluded. †Three or less missing. §Data available for 38.

BD, below detection; mIgG, mouse allergen-specific IgG.

Table 4. Mouse-specific antibody levels and mouse-related symptoms

	Mouse-related symptoms	
	OR (95% CI)	Adjusted OR (95% CI)
mIgE (kU _A /L, log ₁₀)	4.5 (1.6–12.8)	9.9 (2.2–45.0)**
mIgG (log ₁₀)	1.0 (0.5–2.0)*	0.9 (0.5–1.8)†
mIgG4 (log ₁₀)	1.0 (0.3–3.3)*	1.1 (0.3–4.2)†
IgG : IgE (log ₁₀)	1.0 (0.5–2.0)	0.9 (0.4–1.9)**
IgG4 : IgE (log ₁₀)	0.4 (0.2–1.0)	0.3 (0.1–1.0)**

*Adjusted for mIgE (log₁₀). **Adjusted for sex and current handling of mice.

†Adjusted for mIgE, sex, and current handling of mice.

mIgG, mouse allergen-specific IgG; OR, odds ratio; CI, confidence interval.

Table 5. Mouse-specific antibody levels and mouse-related symptoms among mIgE-positive workers

	Mouse-related symptoms	
	OR (95% CI)	Adjusted OR (95% CI)
mIgE (kU _A /L, log ₁₀)	2.5 (0.6–10.4)	6.0 (0.9–42.0)**
mIgG (log ₁₀)	0.4 (0.1–1.2)*	0.1 (0.02–0.7)†
mIgG4 (log ₁₀)	0.4 (0.1–2.2)*	0.2 (0.02–2.1)†
mIgG : IgE (log ₁₀)	0.4 (0.1–1.3)	0.2 (0.02–0.9)**
mIgG4 : IgE (log ₁₀)	0.4 (0.1–1.1)	0.1 (0.02–0.7)**

*Adjusted for mIgE. **Adjusted for sex and current handling of mice. †Adjusted for mIgE, sex, and current handling of mice.

mIgG, mouse allergen-specific IgG; OR, odds ratio; CI, confidence interval.

that prolonged high-level environmental exposure may result in an increase in mouse-specific IgG and IgG4 that is associated with protection against symptoms. Should these findings be confirmed in prospective studies, mouse-specific IgG or IgG4 levels could be used to discern IgE-sensitized workers who are at high risk from those who are at low risk of developing mouse-related symptoms.

Our findings are consistent with other published reports. For example, one study found that rat-specific IgG4 levels were higher among asymptomatic workers than symptomatic workers [28]. Another group examined the relationship between allergen-specific IgG resulting from environmental exposure and skin test threshold. In that particular study,

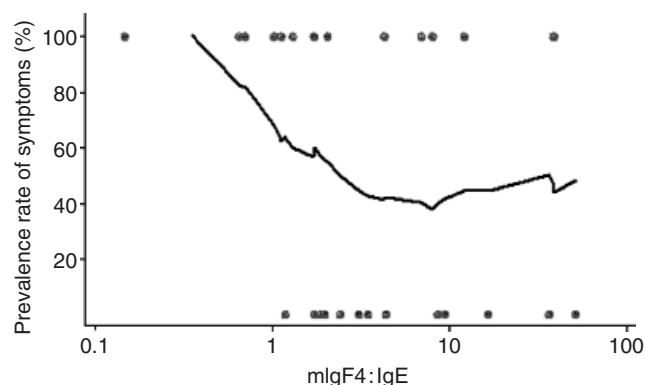


Fig. 1. Estimated probability of mouse-related symptoms among mouse handlers with mouse-specific IgE ≥ 0.35 kU_A/L as a function of mIgG4 : IgE. The solid line represents the estimated prevalence rate of mouse-related symptoms using locally weighted non-parametric smoothing, and the solid circles represent individual data points.

serum levels of cat-specific IgG correlated with the concentration of cat extract required to elicit a positive skin test, suggesting that naturally occurring allergen-specific IgG could play an important role in the induction of clinical tolerance [21]. There have been similar findings in the context of allergen immunotherapy: a study of sublingual grass pollen immunotherapy found that the overall clinical response and the grass-specific IgG4 : gE ratio were positively correlated [15], suggesting that immunologic responses in a laboratory animal setting may be similar to those seen with immunotherapy [14, 15, 17, 29].

On the other hand, our findings differ from those of Portengen et al. [30], who found no relationship between rat-specific IgG4 levels and protection against allergic symptoms, but found that rat-specific IgG4 was a predictor of symptomatic rat allergy. However, the final analyses in this particular study did not account for specific IgE levels. For example, in our study population, mIgG4 and mIgE were moderately correlated, and both associated with mouse-related symptoms. Only after accounting for mIgE level, either by calculating the mIgG4 : IgE ratio, or as a covariate in a multivariable model, did we see a relationship between symptoms and mIgG or mIgG4.

Proposed mechanisms of action of IgG4 include interfering with the binding of IgE to allergen, resulting in an attenuated

late phase skin test response and/or decreased basophil histamine release [18, 29, 31]. In fact, grass pollen-specific IgG4 produced after a course of immunotherapy has been shown to block the formation of allergen-IgE complexes, thereby impeding the processing and presentation of allergen by B cells [19, 29]. Furthermore, increases in IL-10 production by peripheral blood mononuclear cells stimulated with allergen have been shown to correlate with levels of allergen-specific IgG and IgG4, suggesting that T cells exposed to prolonged high doses of allergen are induced to produce IL-10, which, in turn, promotes production of IgG4 [29]. Although T cell cytokine profiles were not examined in the current study, the association of IgG4 with a decreased risk of allergy symptoms is entirely consistent with the findings in immunotherapy studies and supports the notion that clinical tolerance can be achieved through environmental exposure. Future studies of high-level environmental exposure and its effect on immunologic markers should include an examination of allergen-specific T cell responses [15, 21, 28].

Higher mIgG and mIgG4 levels were associated with a decreased risk of mouse-related symptoms among those with mIgE level ≥ 0.35 kU_A/L, but not among the study population as a whole. The reasons for this finding are unclear, although there are several possible explanations. For example, individuals with undetectable mIgE levels, but positive SPTs, may be at such low risk of having symptoms that it would be difficult to detect a protective effect associated with IgG or IgG4, particularly in a relatively small study. These findings may also suggest that a threshold level of IgE-mediated sensitization is necessary before IgG and IgG4 become important predictors of clinical tolerance.

It is also possible that the group with positive SPTs, but undetectable mIgE levels, has IgE to a component of the skin test extract (mouse epithelia) that is not present in the extract substrate used for the RASTs (mouse urine). If this is indeed the case, then mouse urine-specific IgG and IgG4 would have little bearing on the symptomatic status of an individual without specific IgE to mouse urine, and could explain why the protective effect of mIgG and mIgG4 was not seen when workers with mIgE levels < 0.35 kU_A/L were included in the analysis.

Regardless of the underlying reason for the lack of effect among participants with undetectable mIgE levels, the fact that such a strong effect on symptoms was observed in the group with detectable mIgE, for all measures of mouse-specific IgG and IgG4, is compelling evidence that environmentally induced mouse-specific IgG and IgG4 may be associated with clinical tolerance. Larger, prospective studies are needed to explore these relationships further.

The results of this study should be considered in view of some study limitations. For example, our findings could have resulted from selection bias that can occur when symptomatic workers leave jobs because exposure in the current job elicits symptoms. This environmental pressure can result in a selected population of less symptomatic participants who tolerate higher levels of exposure, and who also have higher IgG and IgG4 levels because of the higher exposure. If this type of selection bias had occurred, one would expect that higher levels of exposure would be associated with a decreased risk of symptomatic mouse allergy, but our analyses do not suggest that such an association exists. The

limitations of a cross-sectional study should also be considered. Although we have found a strong association between higher mIgG and mIgG4 levels and a decreased risk of allergy symptoms in IgE-sensitized workers, a prospective study is needed to examine the temporal relationships between these immunologic markers, T cell responses, and mouse allergy symptoms.

In summary, these findings suggest that mouse-specific IgG and IgG4 are associated with a decreased risk of mouse-related symptoms among workers with detectable mIgE. These results indicate that clinical tolerance, as observed with allergen immunotherapy, may also occur through environmental exposure.

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