

Both the variability and level of mouse allergen exposure influence the phenotype of the immune response in workers at a mouse facility

Roger D. Peng, PhD,^a Beverly Paigen, PhD,^b Peyton A. Eggleston, MD,^c Karol A. Hagberg, RN,^b Mary Krevans, RN,^b Jean Curtin-Brosnan, MA,^c Cristy Benson,^b Wayne G. Shreffler, MD, PhD,^d and Elizabeth C. Matsui, MD, MHS^c

Baltimore, Md, Bar Harbor, Me, and Boston, Mass

Background: The role of natural aeroallergen exposure in modulating allergen-specific immune responses is not well understood.

Objective: We sought to examine relationships between mouse allergen exposure and mouse-specific immune responses.

Methods: New employees (n = 179) at a mouse facility underwent repeated assessment of mouse allergen exposure, skin prick tests (SPTs), and measurement of mouse-specific IgG levels. Relationships between the mean level of exposure, variability of exposure (calculated as log deviation), and time to development of immunologic outcomes were examined by using Cox proportional hazards models.

Results: By 24 months, 32 (23%) participants had experienced a positive SPT response, and 10 (8%) had mouse-specific IgG₄. The incidence of a positive SPT response increased as levels of exposure increased from low to moderate, peaking at 1.2 ng/m³, and decreased beyond this point (P = .04). The more variable the exposure was across visits, the lower the incidence of a positive SPT response (hazard ratio [HR], 0.17; 95% CI, 0.07–0.41). Variability of exposure was an independent predictor of a positive SPT response in a model that included both exposure metrics. In contrast, the incidence of mouse-specific IgG₄ increased with increasing levels of mouse allergen exposure (HR, 2.9; 95% CI, 1.4–6.0), and there was evidence of a higher risk of mouse-specific IgG₄ with greater variability of exposure (HR, 6.3; 95% CI, 0.4–95.2).

Conclusion: Both the level and variability of mouse allergen exposure influence the humoral immune response, with specific patterns of exposure associated with specific immunophenotypes. Exposure variability might be a more

important predictor of a positive SPT response, whereas the average exposure level might be a more important predictor of mouse-specific IgG₄. (J Allergy Clin Immunol 2011;■■■:■■■–■■■.)

Key words: Mouse allergen, IgE, IgG₄, laboratory animal allergy

In recent years, our understanding of how natural animal allergen exposure modulates the humoral immune response has grown.^{1–9} However, the relationship between allergen exposure and adaptive immune responses is quite complex, and many questions remain. Allergen exposure has generally been conceived of as intensity or concentration of exposure, but other aspects of exposure also might influence the allergen-specific immune response. For example, the variability of allergen exposure might influence the type of humoral immune response that develops, yet there is little published regarding this relationship. In addition, although the relationship between the intensity or concentration of allergen exposure and immune responses has been examined in many studies, these studies have largely been cross-sectional^{7,10–13}; therefore conclusions from these studies come with many caveats. Addressing these questions about the relationship of exposure and immune response is critical to understanding whether modification of animal allergen exposure is a scientifically supported approach for prevention of allergic sensitization and disease.

Prospective cohort studies with frequent and repeated assessments of exposure and allergic status are essential for gaining insight into the true nature of the exposure-response relationship but are difficult to conduct in community-based populations. On the other hand, an occupational setting is ideal for the intensive prospective assessments required and thus is an appealing model of animal allergen exposure-immune response relationships. We therefore conducted a prospective cohort study of new workers at a mouse research and production facility to test the hypothesis that the level and variability of exposure to mouse allergen influences mouse allergen-specific humoral responses.

METHODS

Study population

As a part of routine procedures at The Jackson Laboratory Health Office, health screening of new employees includes skin prick tests (SPTs) and venipuncture for collection of serum. New employees screened between July 2004 and December 2007 were approached by study staff to determine interest and eligibility for the JAXCohort study. All new, nontemporary, full-time employees at least 18 years of age were eligible to participate. Interested

From ^athe Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore; ^bThe Jackson Laboratory, Bar Harbor; ^cthe Division of Pediatric Allergy and Immunology, Johns Hopkins School of Medicine, Baltimore; and ^dthe Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Boston.

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

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Abbreviations used

AIC: Akaike information criterion
 HR: Hazard ratio
 SPT: Skin prick test

participants were scheduled for a baseline study visit, during which written informed consent was obtained. Consent included permission to use the skin testing results and sera that were collected at their initial health screening visit. The JAXCohort study was approved by institutional review boards at the Johns Hopkins Medical Institutions and The Jackson Laboratory.

Two hundred sixty of 374 eligible new employees consented to a baseline screening visit. One hundred ninety-six met the eligibility criteria for this analysis, which included the following: (1) absence of any evidence of a mouse-specific humoral response at baseline and (2) completion of the first exposure assessment at 6 months. One hundred seventy-nine (90%) of the 196 eligible employees had valid personal mouse allergen exposure data and comprised the population for this analysis.

Clinical assessments

Skin testing was performed at baseline and every 6 months. SPTs were performed to 14 allergens at the baseline visit and 6 allergens at follow-up visits by using the MultiTest II device (Lincoln Diagnostics, Decatur, Ill) with a positive histamine control and a negative glycerol control. The allergens tested at the baseline visit were the following: mouse, rat, cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, pine, birch, oak, orchard grass, *Alternaria* species, *Aspergillus* species, *Penicillium* species, and ragweed. The allergens tested at the follow-up visits were the following: mouse, rat, cat, dog, dust mite mix, and pine. A positive skin test response was defined as an orthogonal wheal size of 3 mm or greater than that elicited by the negative control.

Venipuncture was performed at baseline and every 6 months. Mouse-specific and total IgE levels were measured by using the quantitative ImmunoCAP (Phadia, Uppsala, Sweden). A value of 0.10 kU_A/L or greater for mouse-specific IgE was considered positive. Levels of mouse-specific IgG and IgG₄ were measured in serum samples by using a solid-phase antigen-binding assay, as previously described.¹⁴ The limit of detection for the mouse-specific IgG assay was 20 AU/mL, and that for the mouse-specific IgG₄ assay was 15 AU/mL (1 AU is approximately equivalent to 1.75 ng). Because the mouse allergen-specific IgG assay detects all IgG isotypes, a mouse-specific IgG₁₋₃ response was defined as an IgG response that did not include IgG₄.

The baseline questionnaire was administered by study staff and covered demographic information, pulmonary and allergic history, smoking history, and occupational and family history. The follow-up questionnaire was administered every 6 months and captured interval allergic history and occupational history.

Exposure assessments

Airborne monitoring to assess breathing-zone mouse allergen exposure was conducted starting at the 6-month study visit and every 6 months thereafter. Personal air samples were collected during 2 full 8-hour shifts within a 1-week time period once every 6 months by using Buck VSS-12 personal sampling pumps (A. P. Buck, Inc, Orlando, Fla) with flow rates of 2 L/min. The mean of these 2 measurements was used to represent the average exposure for the worker. Protein was extracted from the filters by using standardized procedures.^{15,16} Mus m 1, the major mouse allergen, was quantified immunochemically by using a sandwich ELISA.^{17,18} Mouse allergen exposure data were considered invalid if there was evidence of pump malfunction or compromise of the filter (eg, a torn filter).

Statistical analyses

The data were first examined by using summary statistics and plots, such as histograms and scatter plots, to check for nonnormal distributions and to

explore bivariate relationships. Because the mouse allergen data were highly right skewed, a log transformation was undertaken, and all subsequent analyses were conducted with the log-transformed data.

Cox proportional hazards models were used to assess the relationship between mouse allergen exposure and a positive SPT response, with the outcome being the time until development of a positive SPT response. The same modeling approach was used to estimate the relative risk of development of mouse-specific IgG₁₋₃ and IgG₄. A participant was considered to have mouse-specific IgG₁₋₃ if he or she had detectable mouse-specific IgG but undetectable mouse-specific IgG₄. A participant was considered to have mouse-specific IgG₄ if he or she had detectable mouse-specific IgG₄. Natural splines were used to estimate potential nonlinear exposure-response functions for each of the 3 outcomes, and partial likelihood ratio tests were used to test for statistically significant nonlinear relationships. For each analysis, the proportional hazards assumption was checked by using scaled Schoenfeld residuals.¹⁹ Joint models estimating the combined effect of long-term variability and mean level of exposure were fit by including both exposure metrics into the model simultaneously. Goodness of fit of the joint model relative to the single exposure models was assessed with the Akaike information criterion (AIC), with smaller AIC values indicating better-fitting models.

Two different metrics of exposure to mouse allergen were used in the statistical analyses: (1) the level of exposure represented by the mean log₁₀ Mus m 1 level across all visits preceding the development of the outcome (eg, positive SPT response or presence of IgG₁₋₃ or IgG₄) and (2) the long-term variation of exposure represented by the log₁₀ deviation of log₁₀ Mus m 1 levels across visits preceding development of the outcome. The first metric measures the typical level of Mus m 1 to which each subject was exposed, and the second measures the longer-term variability of Mus m 1 exposure across study visits (eg, 6 and 12 months). At least 2 visits were required for each subject to calculate the measure of long-term variability ($n = 123$). For all analyses, a 2-tailed P value of less than .05 was considered statistically significant. All analyses were performed with R version 2.11.1 software (R Development Core Team, 2010).

RESULTS**Study population**

The 179 members of the JAXCohort study were predominantly white, with a slight preponderance of female subjects and a mean age of 32 years. Prevalences of reported current asthma (10%) and ever smoking (45%) were similar to other US adult populations.²⁰⁻²² Fifty-one percent had at least 1 positive skin test response to a panel of aeroallergens at baseline, with 35% having positive responses to dust mite, 16% to cat, and 31% to pollens (Table I). The study population included animal caretakers, administrative/support personnel, scientists, laboratory technicians, and materials/supplies handlers; 61% of the population handled mice (Table I). Of the 179 subjects analyzed, 43 had worked with mice before joining The Jackson Laboratory.

The study lasted 42 months; the median time each employee was followed was 23 months (25th-75th percentile, 17-30 months). Seventy-two participants left The Jackson Laboratory during the study, and on average, those who left had higher mean exposure (1.16 ng/m³) to mouse allergen while enrolled in the study compared with those who stayed (0.55 ng/m³, $P = .06$). However, of the 64 (89%) who participated in an exit interview, only 1 reported leaving employment because of mouse allergy.

Mouse allergen exposure

Every 6 months, participants underwent 2 days of personal air monitoring to measure mouse allergen exposure. For each 6-month assessment, the mean of the first- and second-day measurements was calculated for each participant and was taken

TABLE 1. Characteristics of 179 participants

Characteristic	No. (%)
Sex, female	96 (53.6)
Race, white	162 (90.5)
Educational attainment	
≤High school graduate	36 (20.1)
Some college	41 (22.9)
College graduate	50 (27.9)
Postgraduate work	52 (29.1)
Smoking status	
Current smoker	45 (25.1)
Former smoker	35 (19.6)
Never a smoker	99 (55.3)
Allergic history	
Asthma ever	18 (10.1)
Hay fever ever	26 (14.5)
Sensitization/atopic characteristics	
Total IgE (kU/L), median (25th-75th percentile)	13 (5.2-35.8)
Atopic (≥1 positive SPT response)	91 (50.8)
Skin test sensitivity	
Cat	29 (16.2)
Dog	2 (1.1)
Dust mite	63 (35.3)
Pollen	56 (31.3)
Mold	16 (8.9)
Job category	
Animal caretaker	66 (36.9)
Administrative/support staff	40 (22.3)
Scientist	33 (18.4)
Laboratory technician	21 (11.7)
Materials/supplies handler	16 (9.0)
Other	3 (1.7)

as an estimate of mouse allergen exposure in the previous 6 months. This 2-day mean mouse allergen concentration was averaged across visits for each participant. For the entire study, the average number of exposure assessments per participant was 2.8, with a range of 1 to 7 assessments. The median of the average mouse allergen concentration across visits was 0.69 ng/m³ (25th-75th percentile, 0.09-9.88 ng/m³). The median long-term log₁₀ deviation was 0.51 (25th-75th percentile, 0.29-0.98 ng/m³), so that on average, subjects typically experienced a 3-fold range of variation in their exposure concentrations across their follow-up period. Long-term variability tended to be larger among materials handlers and scientists (≥10-fold range in exposure) and smaller among animal caretakers (2-fold variation in exposure, Fig 1).

Mouse skin test sensitivity

By 24 months, 23% (95% CI, 15% to 30%) of participants had a positive SPT response. The risk of a positive mouse SPT response was nonlinear, increasing from low to moderate levels of exposure, peaking at approximately 1.2 ng/m³, and then decreasing from moderate to high levels of exposure ($P = .04$; Fig 2, A). The analyses of long-term variability of exposure included 123 participants with 2 or more exposure measures. This subset did not differ from the larger study population in terms of population characteristics or mouse allergen exposure (see Table E1 in this article's Online Repository at www.jacionline.org). For long-term variability of exposure, the risk of a positive mouse SPT response decreased with increasing long-term variability in mouse allergen exposure (hazard ratio [HR], 0.17; 95% CI, 0.07-0.41; Fig 2, B). We conducted a sensitivity analysis by examining the

subset of participants who had not worked with mice before joining The Jackson Laboratory. In this subset the relationships between mean mouse allergen and positive SPT responses and long-term variability and positive SPT responses were similar to the main analysis. Among the participants with a positive SPT response, 7 also had rhinoconjunctival symptoms (sneezing, watery eyes, and runny nose) or lower respiratory tract symptoms (cough, wheezing, and chest tightness).

Mouse-specific IgG₄ and IgG₁₋₃

By 24 months, 8% (95% CI, 3% to 16%) of participants had mouse-specific IgG₄. In models using only 1 of the exposure measures, high levels of mouse allergen exposure were positively associated with the development of mouse-specific IgG₄ (HR, 2.86; 95% CI, 1.39-5.86), but variability of exposure was not (HR, 0.83; 95% CI, 0.16-4.32; Fig 3). By 24 months, 10% (95% CI, 5% to 15%) of participants had mouse-specific IgG₁₋₃. Although higher average exposure and lower variability were associated with development of mouse-specific IgG₁₋₃, these relationships were not statistically significant (see Tables E5 and E6 in this article's Online Repository at www.jacionline.org).

Combined effects of variability and level of exposure

The combined effects of long-term variability and mean level of exposure were modeled by using Cox proportional hazard models (Fig 4). The highest risk of skin test sensitivity was observed with low variability and a moderate level of exposure (Fig 4, A), but only long-term exposure variability remained an independent predictor of incident skin test sensitivity. Mouse-specific IgE levels were correlated with mouse skin test sensitivity (correlation coefficient = 0.44, $P < .01$) but was a more rare event than skin test sensitivity, with 5% of participants having mouse-specific IgE by 24 months. Relationships between average exposure and long-term variability of exposure and mouse-specific IgE levels were consistent with those observed for skin test sensitivity but were not statistically significant (see Fig E1 in this article's Online Repository at www.jacionline.org).

The highest risk of mouse-specific IgG₄ in the models for combined effects was observed with high variability and high-level exposure (HR of 6.34 [95% CI, 0.42-95.19] and HR of 3.59 [95% CI, 1.71-7.53, respectively; Fig 4, B), but only the level of exposure remained an independent predictor of mouse-specific IgG₄. However, this joint model for risk of IgG₄ was considered by the AIC to be superior to models, including mean exposure or variability alone. Relationships observed between the exposure metrics and a positive SPT response and mouse-specific IgG₄ levels were robust to adjustment for potential confounders, including age, sex, smoking status, total IgE levels, and respiratory protection use (see Tables E2, E3, and E4 in this article's Online Repository). In addition, atopy, which has been shown to be a risk factor for sensitization and allergen-specific IgG₄ in previous studies,^{8,23,24} was also a predictor of these outcomes in this study population (see this article's Online Repository).

Skin test sensitivity and mouse-specific IgG responses

Most study participants had neither skin test sensitivity nor mouse-specific IgG₄. Of the 42 participants who had either a

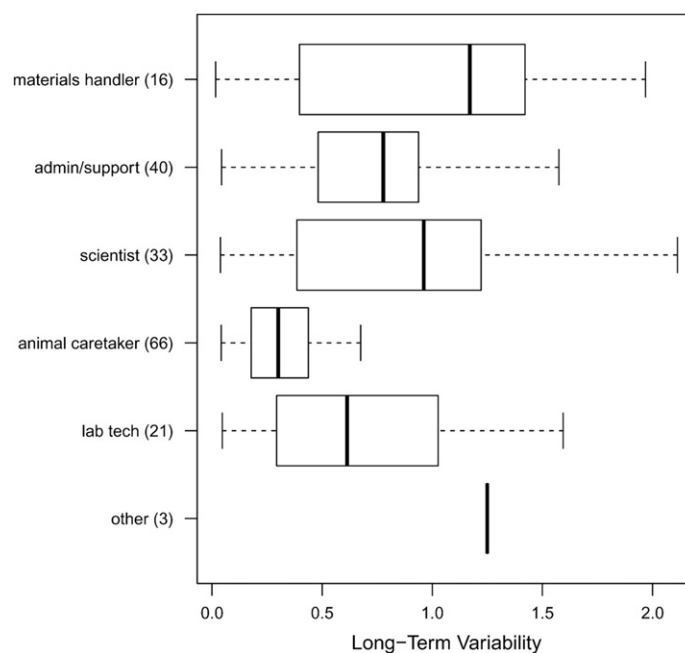


FIG 1. Box plots of long-term variability of mouse allergen exposure (expressed as the log₁₀ [deviation of exposure]) by job category. Each box plot indicates the minimum, 25th percentile, median, 75th percentile, and maximum of the data.

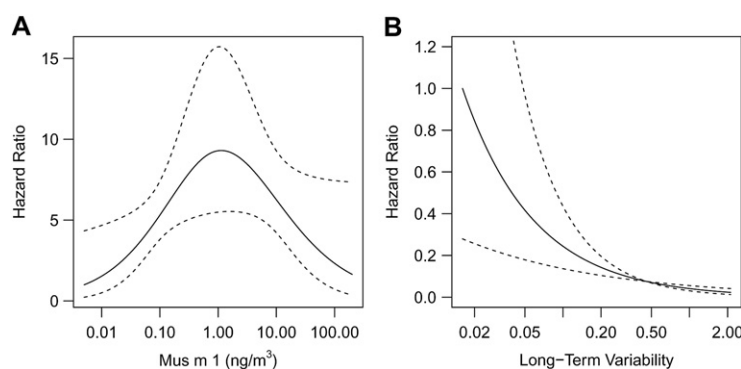


FIG 2. Plots depicting relationships between level of exposure (**A**) and long-term variability of exposure (**B**) and positive SPT responses. The relationships predicted by the statistical models are indicated by *solid lines*, and 95% CIs are indicated by *dotted lines*.

positive skin test response or mouse-specific IgG₄, only 2 had both skin test sensitivity and IgG₄, and the remaining 40 had either skin test sensitivity or mouse-specific IgG₄ but not both, suggesting that these 2 immune responses are inversely related to one another. A similar pattern was observed for skin test sensitivity and mouse-specific IgG₁₋₃.

DISCUSSION

In this occupational JAXCohort study, both the level and variability of mouse allergen exposure were associated with the pattern of mouse-specific immune responses. A pattern of stable moderate exposure was most strongly associated with the development of allergic sensitization; a pattern of variable high-level exposure was most strongly associated with an IgG₄ response. Furthermore, long-term exposure variability was the more important predictor of skin test sensitivity, whereas the level of exposure

was the more important predictor of mouse-specific IgG₄ levels. These findings extend previous work by highlighting the potential role of exposure variability in modulating the risk of allergic sensitization, as well as the capacity of multiple characteristics of exposure to simultaneously influence the immune response.

These findings have potentially important implications for primary and secondary prevention of allergic respiratory disease. For primary prevention, tailoring job duties or career paths to avoid moderate stable exposure might help reduce the incidence of sensitization. For secondary prevention, our findings support the notion that high-level exposure might steer an allergic immune response toward an IgG₄ response, thus providing a rationale for evaluating the role of immunotherapy in preventing the development of allergic disease in workers with early evidence of an allergic immune response.

Our findings are consistent with observations made on the humoral response to other allergens in healthy adult populations.

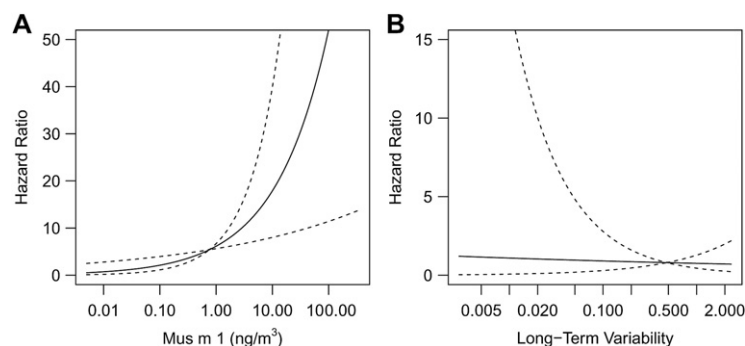


FIG 3. Plots depicting relationships between level of exposure (**A**) and long-term variability of exposure (**B**) and mouse-specific IgG₄ levels. The relationships predicted by the statistical models are indicated by *solid lines*, and 95% CIs are indicated by *dotted lines*. Long-term variability is expressed as the $\log_{10}$ (deviation of exposure).

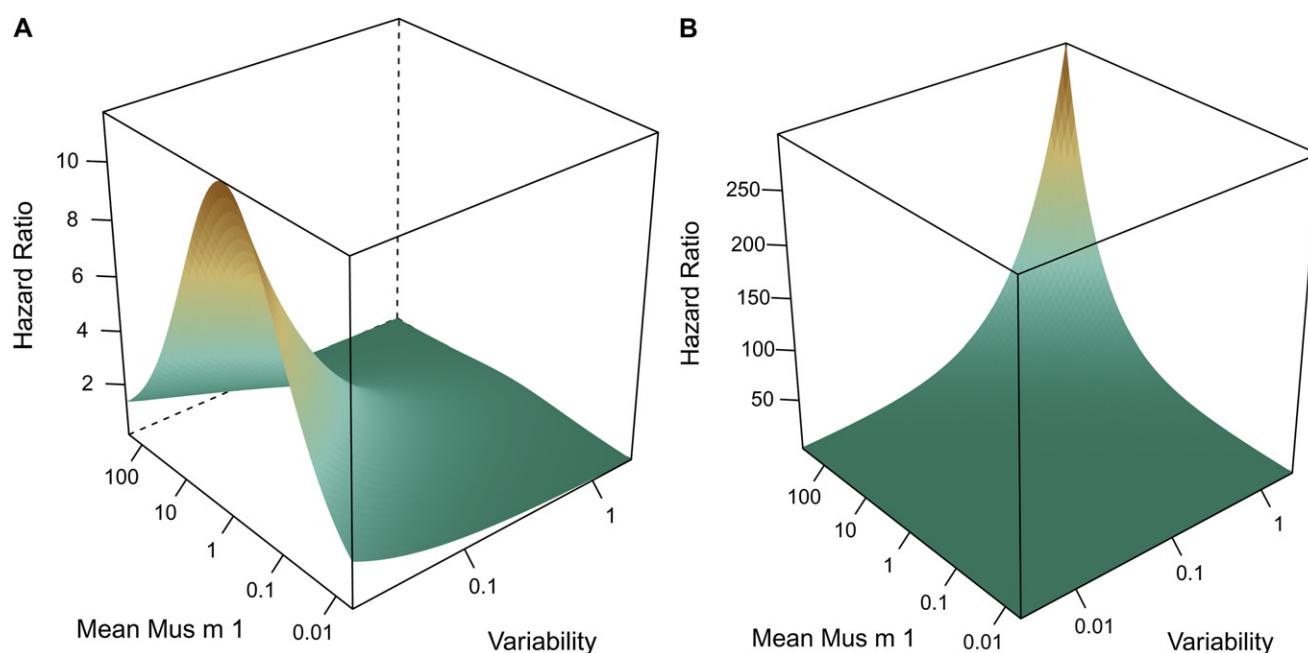


FIG 4. Surface plots depicting relationships between level of exposure, variability of exposure, and risk of a positive SPT response (**A**) and mouse-specific IgG₄ levels (**B**). Long-term variability is expressed as the $\log_{10}$ (deviation of exposure).

For example, beekeepers, who receive intermittent stings separated by periods of no exposure, have a pattern of variable high-level exposure and primarily mount an IgG₄ rather than an IgE response to the honeybee antigens.²⁵ This finding is also consistent with work demonstrating that the development and regulation of IgE follows a unique program that is associated with class switching outside of germinal centers and often involves sequential $\mu \rightarrow \gamma \rightarrow \epsilon$ class switching.²⁶ Both poor germinal center development and IgE class switching are favored during chronic exposure of weakly proinflammatory antigens,²⁷ as experienced by those with stable moderate dose exposure in this cohort. This observation also suggests that exposure variability might influence the T-cell response, such that high-dose variable exposure might favor the development of IL-10, which has effects on class switching, plasma cell differentiation, and survival, favoring IgG₄ production over IgE production.²⁸

Some previous studies of mouse allergen exposure are consistent with our findings from the JAXCohort study. In a cross-sectional study Jeal et al¹⁰ reported that high rat exposure (>50 rats handled per day) was associated with a higher risk of IgG and IgG₄ responses and, in contrast, a lower risk of an IgE response and allergic symptoms. Results from other occupational studies,^{6,7,29} including a previous cross-sectional study at The Jackson Laboratory,¹³ differ from results of the JAXCohort study, generally finding a monotonically increasing relationship between various exposure metrics and risk of sensitization (and IgG or IgG₄ for some studies). In a recent study a higher number of mouse contact hours per month was associated with a greater risk of sensitization, but there was no relationship between this exposure metric and mouse-specific IgG₄ levels.³⁰ In this study participants all handled mice and could have worked at the facility for as long as 18 months at the time of enrollment. Not

surprisingly, a significant proportion of participants had mouse-specific IgG₄ at enrollment. In contrast, our study included new workers (including non-mouse handlers) with no evidence of a mouse-specific humoral responses, so that our findings pertain to exposure-response relationships during an earlier time period in the development of allergen-specific immune responses. Other likely explanations for the apparent discrepancies between some of these other studies and ours include potential bias because of cross-sectional study designs, estimated versus measured allergen exposure, and differences in exposure metrics.^{6,7} It is also important to note that many of these other studies reported attenuated risk of some outcomes at the highest levels of exposure, which was sometimes attributed to survival bias.^{7,29,31} In our study, however, there is no evidence to suggest that the findings are a result of survival bias (specifically the healthy worker effect). We interviewed 89% of the participants who left the study, and only 1 participant reported leaving because of mouse allergy. Although it is possible that workers modify their behavior to reduce exposure when allergic symptoms develop, only a very small number of sensitized participants also had mouse-associated allergic symptoms, and therefore this is unlikely to have biased our results.

Although occupational mouse allergen exposure does not perfectly mimic community exposure, the occupational setting can serve as a model of allergen exposure-immune response relationships. Results of community-based studies of allergen exposure-immune response relationships have been mixed. For example, birth cohort studies have found an increasing prevalence of IgG responses to cat over time³² and dose-dependent relationships between cat allergen exposure and cat-specific IgE and IgG₄ responses.³³ In contrast, 1 study of mouse allergen exposure and mouse-specific humoral responses in inner-city preschool children suggested that children with the highest levels of home exposure were less likely to have both sensitization and mouse allergen-specific IgG responses than children with moderate levels of exposure.¹¹ Combined with the results of the JAXCohort study, there is evidence to support further studies of mouse allergen exposure and immune responses in community populations, with the objective of determining whether immune biomonitoring and early immunotherapy are viable research avenues to pursue.

Although our occupational setting can serve as a model for mouse allergen exposure in other settings, we acknowledge 2 limitations related to the study population and immune response evaluation. Our findings cannot be directly applied to populations that differ from our healthy, predominantly white, worker population. Although the temporal relationships between our measures of exposure and skin test sensitivity and IgG₄ outcomes provide strong evidence for a causal relationship between long-term variability and level of exposure and allergen-specific humoral responses, cellular immune responses were not evaluated, and therefore no conclusions can be drawn regarding the possible cellular immune mechanisms that might be at play.

This prospective JAXCohort study confirmed the findings of previous cross-sectional studies that high-level rodent allergen exposure protects against allergic sensitization while promoting allergen-specific IgG₄ responses. More importantly, this is the first epidemiologic study, to our knowledge, to find that different characteristics of exposure, namely variability and level of exposure, can together influence the allergen-specific immune response. Our findings support further examination of the utility of immune biomonitoring and immunotherapy in secondary prevention of rodent allergy.

Key messages

- In this occupational cohort a pattern of stable moderate exposure was most strongly associated with the development of allergic sensitization. A pattern of variable high-level exposure was most strongly associated with an IgG₄ response.
- Because high-level exposure might steer an allergic immune response toward an IgG₄ response, allergen immunotherapy should be studied as a possible preventive measure for mouse allergy.

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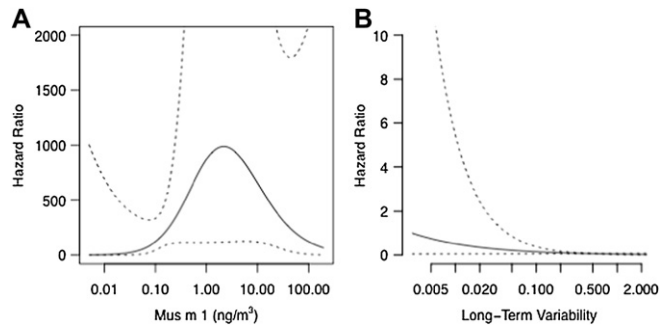


FIG E1. Plots depicting relationships between level of exposure (**A**) and long-term variability of exposure (**B**) and mouse-specific IgE levels. The relationships predicted by the statistical models are indicated by *solid lines*, and 95% CIs are indicated by *dotted lines*.

TABLE E1. Characteristics of participants with at least 2 exposure assessments (n = 123)

Characteristic	No. (%)
Sex, female	64 (52.0)
Race, white	112 (91.1)
Educational attainment	
≤High school graduate	21 (17.1)
Some college	25 (20.3)
College graduate	38 (30.9)
Postgraduate work	39 (31.7)
Smoking status	
Current smoker	29 (23.5)
Former smoker	23 (18.7)
Never a smoker	71 (57.7)
Allergic history	
Asthma ever	11 (9.9)
Hay fever ever	21 (17.1)
Sensitization/atopic characteristics	
Total IgE (kU/L), median (25th-75th percentile)	10.3 (4.7-35.8)
Atopic (≥1 positive SPT response)	59 (48.0)
Skin test sensitivity	
Cat	18 (14.6)
Dog	1 (0.8)
Dust mite	39 (31.7)
Pollen	40 (32.5)
Mold	13 (10.6)
Job category	
Animal caretaker	38 (30.9)
Administrative/support staff	32 (26.0)
Scientist	25 (20.3)
Laboratory technician	16 (13.0)
Materials/supplies handler	11 (8.9)
Other	1 (0.8)

TABLE E2. Multivariate Cox proportional hazards regression model of the relationship between average mouse allergen exposure and time to development of mouse skin test sensitivity, adjusting for age, sex, atopy, smoking status, and respiratory protection use

	Log hazard ratio	Hazard ratio	Standard error	z Value	P value
Age (y)	−0.03	0.97	0.02	−1.31	.19
Sex, male	0.01	1.01	0.40	0.04	.97
Atopy (≥ 1 positive SPT response)	1.23	3.43	0.46	2.69	.01
Current smoker	−0.11	0.89	0.46	−0.24	.81
Respiratory protection, sometimes*	−0.77	0.46	1.03	−0.74	.46
Respiratory protection, always*	−0.12	0.88	0.51	−0.25	.81
Average mouse allergen exposure (ng/m^3), spline term 1	3.20	24.62	1.83	1.75	.08
Average mouse allergen exposure (ng/m^3), spline term 2	−0.90	0.41	1.01	−0.90	.37

*The reference group is “respiratory protection use, never.”

TABLE E3. Multivariate Cox proportional hazards regression model of the relationship between long-term variability of mouse allergen exposure and development of mouse skin test sensitivity, adjusting for age, sex, atopy, smoking status, and respiratory protection use

	Log hazard ratio	Hazard ratio	Standard error	z Value	P value
Age (y)	−0.04	0.96	0.03	−1.39	.16
Sex, male	−0.09	0.91	0.57	−0.16	.87
Current smoker	−0.13	0.88	0.59	−0.22	.83
Atopic (≥ 1 positive SPT response)	1.70	5.47	0.76	2.23	.03
Respiratory protection, sometimes*	−16.68	0.00	6916.26	−0.00	1.00
Respiratory protection, always*	0.89	2.43	0.68	1.31	.19
Long-term variability of mouse allergen exposure†	−2.61	0.07	0.65	−4.02	.00

*The reference group is "respiratory protection use, never."

†Log₁₀ of the deviation of mouse allergen exposure.

TABLE E4. Multivariate Cox proportional hazards regression model of the relationship between average mouse allergen exposure and development of mouse-specific IgG₄, adjusting for age, sex, smoking status, atopy, and respiratory protection use

	Log hazard ratio	Hazard ratio	Standard error	z Value	P value
Age (y)	−0.19	0.83	0.09	−2.03	.04
Sex, male	−0.04	0.96	0.92	−0.05	.96
Current smoker	3.05	21.05	1.61	1.90	.06
Atopic (≥1 positive SPT response)	3.96	52.41	1.76	2.25	.02
Respiratory protection, sometimes*	−17.24	0.00	14,592.69	−0.00	1.00
Respiratory protection, always*	−1.06	0.35	1.15	−0.92	.36
Average mouse allergen	0.95	2.57	0.46	2.04	.04

*The reference group is “respiratory protection use, never.”

TABLE E5. Cox proportional hazards regression model of the relationship between average mouse allergen exposure and development of mouse-specific IgG₁₋₃

	Log hazard ratio	Hazard ratio	Standard error	z Value	P value
Average mouse allergen	0.48	1.62	0.32	1.51	.13

TABLE E6. Cox proportional hazards regression model of the relationship between average mouse allergen exposure and development of mouse-specific IgG₁₋₃

	Log hazard ratio	Hazard ratio	Standard error	z Value	P value
Long-term variability of mouse allergen exposure*	−0.97	0.38	0.72	−1.34	.18

*Log₁₀ of the deviation of mouse allergen exposure.