Modified Th2 Responses at High-Dose Exposures to Allergen

Using an Occupational Model

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Rationale: **The relationships between allergen exposures and allergy and asthma are complex. High exposure levels to cat allergen are associated with IgG- and IgG_4-specific antibody responses without sensitization or risk of asthma, a process described as a "modified Th2 response." Attenuation of risk of allergy and asthma at high exposure levels has been reported in longitudinal studies of both childhood and occupational asthma.**

Objectives: **To investigate, using an occupational model, the relationships among estimated exposure to aeroallergens, the production of specific IgE, IgG and IgG_4 antibodies, and the prevalence of associated symptoms.**

Methods: **Cross-sectional survey of employees exposed to rats at work on six pharmaceutical sites across the United Kingdom. A total of 689 (89%) provided a blood sample and completed a questionnaire.**

Measurements and Main Results: **At highest exposure to rats, there was an attenuation of the exposure response for sensitization and symptoms. In contrast, the frequency of individuals producing high quantities of specific IgG and IgG_4 increased with exposure intensity. Ratios of IgG_4/IgE were highest in those handling the greatest number of rats. Risk of developing work-related chest symptoms was lower for those who produced both specific IgE and IgG_4 compared to those with specific IgE only.**

Conclusions: **High exposure to rats is associated with lower rates of specific IgE and symptoms but an increased frequency of high specific IgG and IgG_4 production. Specific IgG_4 produced together with specific IgE may reduce the risk of developing work-related chest symptoms compared with when specific IgE is produced alone.**

Keywords: IgG; IgG_4; IgE; laboratory animal allergy; occupational allergy

The relationship between allergen exposures and the induction of allergy and asthma is complex. On cross-sectional analysis, high current exposures to cat allergens are associated with specific IgG and IgG_4 antibody responses in the absence of specific sensitization or risk of asthma, a state that has been described as a "modified Th2 response" (1). We have reported high-dose attenuation in a prospectively studied cohort investigating the relationship between early allergen exposure and childhood allergy (2). The risks of IgE sensitization and atopic wheeze at age 5.5 yr increased at very low levels of exposure to cat (and house dust mite) allergen but were attenuated thereafter.

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Occupational asthma, such as caused by laboratory animal allergy (LAA), provides a useful model of allergic asthma because its phenotypes are well defined and exposure can be readily characterized and measured. Again, we have observed in a prospective study of a cohort of laboratory animal workers an attenuation at high dose of the relationships between exposure to rat urinary proteins and specific IgE antibodies (3).

The findings of previous studies of specific IgG antibodies in LAA have been difficult to reconcile. In some, the presence of specific IgG antibodies was significantly associated with intensity, but not duration, of exposure to rats (4, 5); in others, cumulative exposure (to mice) was associated with high specific IgG production (6). A protective role for specific IgG_4 was suggested by one study in which symptomatic individuals, with rat-specific IgE, had lower titers of IgG_4 than corresponding asymptomatic, IgE-positive subjects (7). More recently, the longitudinal relationship between specific IgG_4 and rat allergy was examined using information obtained over 2 yr of follow-up. High levels of specific IgG_4 antibodies to rat urine were a strong predictor of prevalent and incident sensitization and symptomatic rat allergy in atopic and rat-sensitized subjects (8).

We carried out this study to help us understand the basis for our previous longitudinal findings (3). Using a cross-sectional design, we have investigated the relationships among workplace exposure to rat aeroallergens, the production of specific antibodies (IgE, IgG, and IgG_4), and the prevalence of associated symptoms.

METHODS

Subjects

We surveyed 776 employees of six U.K. pharmaceutical companies who were undergoing routine health surveillance for LAA. Employees were eligible for this analysis if they had been exposed to rat proteins at work for at least 1 mo (n = 718). All were invited to complete a questionnaire and undergo skin-prick tests and venesection. Nineteen employees of Imperial College who had never had exposure to rats were also studied. Serum antibodies were measured in 689 (96%) of the 718 eligible employees and all 19 unexposed individuals.

The Royal Brompton Hospital/National Heart and Lung Institute (NHLI) ethics committee approved the study; written, informed consent was obtained from all participants.

Exposure and Symptom Assessment

Dates of first and most recent handling of rats were recorded and the duration of contact with rat proteins estimated: analyses were carried out among the whole population and separately among employees with no more than 3 yr of exposure. Employees were classified according to the job they had ever had that incurred the highest exposure to rats—office or maintenance worker (low exposure), scientist (medium), or animal technician or cage cleaner (high) (9)—and also by the maximum number of rats they had ever handled in 1 d (none, 1–10, 11–50, and 50+). The questionnaire also enquired into eye/nasal and chest symptoms, the latter defined by wheezing, tightness of the chest, or difficulty in breathing. Symptoms were considered to be related to work if they occurred at work or improved when away from work, or both.

Useful information on symptoms was not obtained from 19 pharmaceutical employees.

Atopy

We performed skin-prick tests with histamine, saline, and extracts of cat, grass pollen, and *Dermatophagoides pteronyssinus* (Allergopharma, Reinbek, Germany). Atopy was defined by a positive response (⩾ 3 mm wheal) to tests with one or more of the above extracts. We carried out further prick testing with an extract of rat urine and categorized as a positive response in the same way.

Measurement of Specific IgE

We measured specific IgE to an extract of rat urine using a radioallergosorbent test (RAST) (10). Concordance between RAST and skin-prick tests to rat urine was near complete (96%, $\kappa = 0.78$). RAST results are expressed as the percentage of binding of the total ^{125}I anti-IgE added, with a figure of 2% or greater considered positive.

Measurement of Rat-specific IgG and IgG_4

We measured rat urine–specific IgG and IgG_4 antibodies using an ELISA developed in our laboratory for this project, and in this way recorded an optical density for each antibody measurement. The specificities of the assays were confirmed by an ELISA inhibition assay (additional details on these methods are provided in the online supplement). Rat-specific IgG and IgG_4 optical density values were divided into quartiles and values within the top quartile were considered positive (⩾ 0.46 for IgG, ⩾ 0.34 for IgG_4).

Statistical Analyses

We assessed differences between sensitized and nonsensitized individuals using χ^2 tests, and used χ^2 tests for trend to compare proportions of IgE sensitization and high levels of IgG_4 across exposure categories. Mann-Whitney tests were used to examine differences in IgG, IgG_4, and both IgG:IgE and IgG_4:IgE ratios between groups of sensitized and symptomatic subjects. Associations between specific IgE and IgG_4 and work-related chest symptoms were modeled using multivariate logistic regression, with interaction terms for specific antibodies investigated and reported where significant. Confounding by atopy, exposure, duration of exposure, age, sex, or smoking was considered. All statistical analyses were undertaken using SAS, version 8.02 (SAS Institute, Cary, NC), and Stata, version 7.0 (StataCorp, College Station, TX) statistical software.

RESULTS

Subjects

The employees included in this analysis were, on average, 35 yr old (range, 17–65 yr); 363 (53%) were male. There was a close agreement between the exposure categorization based on job title and that based on the maximum number of rats handled in 1 d (Table 1). Thirty-eight percent had handled more than 50 rats a day, whereas 17% had never handled any; equal proportions (23 and 22%) had handled up to 1 to 10 or 11 to 50 rats. For most (464 = 68%), their highest exposure to rat proteins had been during work as a scientist. Twenty percent (n = 140) had worked in animal husbandry, whereas 12% (n = 81) had only ever worked in low-intensity jobs. These proportions were not greatly different (63, 16, and 22%, respectively) among those employed for 3 yr or less, although the proportion of low-intensity employees was a little higher.

Forty-three percent of all eligible workers were atopic; this proportion varied by exposure category ($p = 0.01$), being lowest among those in the highest intensity job category. We found the same pattern when we restricted the population to those with no more then 3 yr of exposure (Table 1), and when we restricted it further to those with less than 2 yr, 1 yr, and 6 mo of exposure (data not shown).

Antibody Measurements

All employees. Four hundred and three (58%) employees in the analysis had a rat-specific IgE RAST of less than 2% binding and produced levels of rat-specific IgG and IgG_4 below the highest quartile of the distribution. A total of 79 (11%) had specific IgE sensitization to rat urinary proteins (as determined by RAST), a third (28/79, 35%) of whom had high levels of both rat-specific IgG and IgG_4 antibodies. Fifty-nine (9%) had high levels of both specific IgG and IgG_4 antibodies to rat urinary protein in the absence of an IgE response. Rat IgE–sensitised individuals were more often atopic (59/79, 75%) than those who were not sensitized (238/610, 39%), a difference that was statistically significant ($p < 0.001$). Atopic status increased the odds of positive IgE (crude odds ratio [OR], 4.85; 95% confidence interval [CI], 2.82, 8.35); this association remained significant after adjusting for exposure (OR, 4.89; CI, 2.82, 8.46). Approximately 41% (62/152) of employees who reported any work-related symptoms also had rat-specific IgE antibodies, a proportion significantly higher ($p < 0.001$) than among those who did not report such symptoms (3%, 15/518).

When measured on a continuous scale, rat-specific IgG and IgG_4 antibody levels were significantly higher in employees with IgE sensitization to rats and in those with both rat sensitization and work-related chest or eye/nose symptoms (Table 2). Predictably, ratios of rat-specific IgG/IgE and specific IgG_4/IgE were significantly higher in those who were not sensitized to rats and in those without symptoms.

Ninety employees reported work-related nasal or chest symptoms, in the absence of rat-specific IgE sensitization. Their median levels of rat-specific IgG (0.21) and IgG_4 (0.09) and ratios

TABLE 1. EXPOSURE CATEGORIES, DURATION OF EXPOSURE, AND PREVALENCE OF ATOPY AMONG SURVEYED EMPLOYEES

	Maximum Number of Rats Ever Handled in 1 d				
	None	1–10	11–50	50+	Total
All employees					
No.	116	160	151	258	685
Office/maintenance worker (low), n (%)	68 (84)	9 (11)	1 (1)	3 (4)	81
Scientist (medium), n (%)	46 (10)	142 (31)	139 (30)	137 (30)	464
Technician/cage cleaner (high), n (%)	2 (1)	9 (6)	11 (8)	118 (84)	140
Atopy, n (%)	54 (47)	80 (50)	69 (46)	93 (36)	296
Median duration of exposure, yr (range)	3.3 (0.1–24.8)	5.6 (0.2–30.3)	9.1 (0.1–29.8)	12.1 (0.2–41.6)	
Short-duration employees (⩽ 3 yr exposure)					
No.	50	50	26	22	148
Atopy, n (%)	29 (58)	29 (58)	10 (38)	5 (23)	73
Median duration of exposure, yr (range)	1.4 (0.1–2.9)	1.1 (0.1–3.0)	1.3 (0.1–2.9)	1.66 (0.2–2.9)	

* Denominators vary slightly because not all employees replied to all questions.

TABLE 2. SPECIFIC ANTIBODY RESPONSES IN SENSITIZED AND NONSENSITIZED AND SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS*

	n	IgG (OD)	IgG_4 (OD)	IgG/IgE†	IgG_4/IgE†
Total population	689				
IgE+, median (range)	79	0.48 (0–1.34)	0.34 (0–1.34)	0.06 (0–0.51)	0.04 (0–0.44)
IgE−, median (range)	610	0.24 (0–1.67)	0.09 (0–1.43)	0.40 (0–4.95)	0.14 (0.01–4.78)
p		< 0.001	< 0.001	< 0.001	< 0.001
IgE+ with work-related chest symptoms, median (range)	30	0.54 (0.05–1.34)	0.25 (0–1.08)	0.06 (0.0–0.32)	0.03 (0–0.21)
IgE− without work-related chest symptoms, median (range)	575	0.24 (0–1.67)	0.09 (0–1.43)	0.40 (0–4.96)	0.14 (0–4.78)
p		< 0.001	0.004	< 0.001	<0.001
IgE+ with work-related eye/nose symptoms, median (range)	61	0.50 (0–1.26)	0.35 (0–1.03)	0.06 (0–0.51)	0.04 (0–0.38)
IgE− without work-related eye/nose symptoms, median (range)	506	0.24 (0–1.26)	0.09 (0–1.43)	0.41 (0–4.96)	0.15 (0–4.78)
p		< 0.001	0.001	< 0.001	< 0.001
Maximum duration of exposure to rat proteins ⩽ 3 yr	148				
IgE+, median (range)	8	0.51 (0–1.34)	0.18 (0.01–1.08)	0.06 (0–0.16)	0.03 (0–0.20)
IgE−, median (range)	140	0.22 (0–1.43)	0.07 (0–1.1)	0.36 (0–3.33)	0.1 (0–2.62)
p		0.42	0.06	< 0.001	0.17
IgE+ with work-related chest symptoms, median (range)	3	0.67 (0.53–1.34)	0.54 (0.09–1.08)	0.10 (0.06–0.16)	0.08 (0.01–0.13)
IgE− without work-related chest symptoms, median (range)	134	0.22 (0–1.43)	0.07 (0–1.10)	0.35 (0–3.33)	0.10 (0–2.62)
p		0.01	0.05	0.03	0.57
IgE+ with work-related eye/nose symptoms, median (range)	5	0.53 (0–0.67)	0.10 (0.01–0.67)	0.05 (0–0.16)	0.04 (0–0.20)
IgE− without work-related eye/nose symptoms, median (range)	113	0.22 (0–1.43)	0.06 (0–0.94)	0.36 (0–3.33)	0.10 (0–2.39)
p		0.59	0.18	0.001	0.43

* From the total surveyed population (n = 689) and those with a duration of exposure of ⩽ 3 yr (n = 148).
† IgE measured on a continuous scale.

of IgG/IgE (0.33) and IgG_4/IgE (0.12) were not different from those without IgE sensitization to rats and without any work-related symptoms.

Short-duration employees. In general, restriction of our analyses to the 148 individuals with exposure of no more than 3 yr showed similar patterns to those above, although less power was available for analysis (Table 2). The relationships between rat-specific IgG and IgG_4 antibodies and IgE sensitization with eye/nose symptoms were much weaker—and not statistically significant—than they were for chest symptoms.

Unexposed control subjects. No unexposed controls were sensitized to rat urine and the majority had low levels of rat-specific IgG (median, 0.19; range, 0–0.51) and IgG_4 (median, 0.06; range, 0–0.55). Three produced high levels of specific IgG (⩾ 0.46) and four produced high specific IgG_4 (⩾ 0.34).

Antibody Production and Allergen Exposure

Across the lower categories of exposure intensity, there was an increasing prevalence of IgE sensitization to rats (Figure 1); the prevalence in the highest exposure category, however, was reduced. This pattern was apparent in both atopic and nonatopic employees (Figure 1). In contrast, there was a continuous increase in the prevalence of employees with high levels of rat-specific IgG and, separately, rat-specific IgG_4 with increasing exposure (χ^2 tests for trend, $p = 0.003$ and $p < 0.001$, respectively; Figure 2A). Ratios of rat-specific IgG_4/IgE were significantly increased in those handling the highest number of rats ($p < 0.01$; Figure 3).

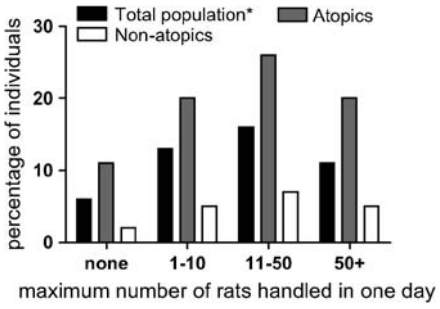

Figure 1. Prevalence of sensitization in the total population and atopic and nonatopic employees. *Denominator populations differ between groups: total population: none (n = 116), 1–10 (n = 160), 11–50 (n = 151), 50+ (n = 258). Atopics: none (n = 54), 1–10 (n = 80), 11–50 (n = 69), 50+ (n = 93). Nonatopics: none (n = 62), 1–10 (n = 80), 11–50 (n = 82), 50+ (n = 164).

Restriction of our analyses to employees of short duration (⩽ 3yr) showed a similar bell-shaped pattern of exposure–response for IgE sensitization to rats (Figure 2B). In contrast, as with the full population, the proportion of individuals producing high levels of rat-specific IgG and IgG_4 was greatest in the highest exposure category. Again, ratios of specific IgG_4/IgE were highest in short-duration employees who had worked with the highest numbers of rats, a difference that was statistically significant ($p = 0.05$).

Work-related Symptoms and Allergen Exposure

In parallel with the reduction in the prevalence of rat-specific IgE sensitization at high exposure, an attenuation of the exposure–response relationship was seen for individuals with work-related chest symptoms who were sensitized to rat protein (Figure 2A). In short-duration employees, a similar pattern was seen: no chest symptoms were reported for short-duration individuals handling more than 11 rats per day (Figure 2B). A similar pattern was seen for work-related eye/nose symptoms (data not shown).

Through multivariate logistic regression analysis, we confirmed the strong relationship between rat-specific IgE sensitization and work-related chest symptoms, and a weaker relationship with atopy. There was a reduction in the risk of work-related chest symptoms among those employees whose rat-specific IgE sensitization was accompanied by high levels of rat-specific IgG_4, compared with those producing specific IgE alone (Table 3). We did not find these relationships when we examined the risk of eye/nose symptoms.

DISCUSSION

Within this cross-sectional examination of laboratory animal workers, we observed complex patterns between exposure to

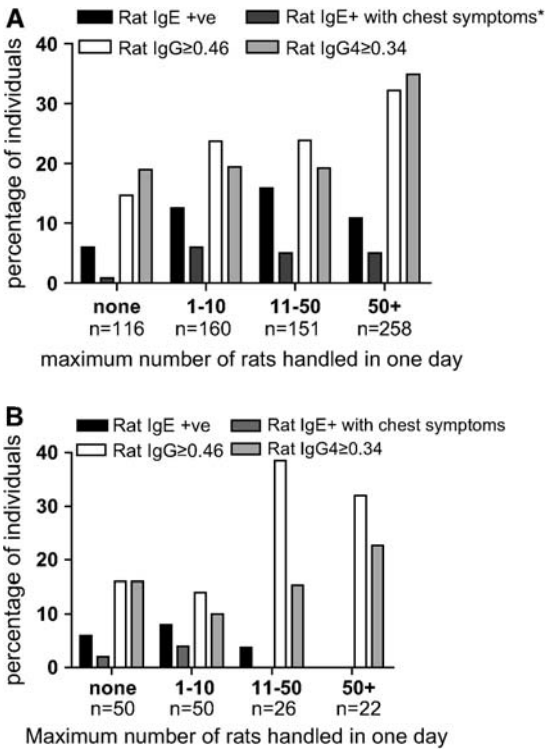

Figure 2. (*A*) Prevalence of sensitization, sensitization with symptoms and IgG (⩾ 0.46), and IgG_4 (⩾ 0.34) antibodies to rat urine stratified by maximum number of rats handled in 1 d. *Numbers for individuals with recorded symptom information differ from the rest of the graph: none (n = 110), 1–10 (n = 157), 11–50 (n = 147), 50+ (n = 254). (*B*) Prevalence of sensitization, sensitization with symptoms, and IgG (⩾ 0.46) and IgG_4 (⩾ 0.34) antibodies to rat urine stratified by maximum number of rats handled in 1 d in short-duration employees.

rats and both sensitization and work-related symptoms. The findings are consistent with those from our previous cohort study (3), whereby there appear to be increasing risks of sensitization and work-related symptoms with increasing exposures to rats, except at the highest exposure levels where the risks of both outcomes may be lower. In parallel, in this study, we found an increase in the prevalence of individuals producing high levels of rat-specific IgG and IgG_4 antibodies with increasing exposure intensity.

We were specifically interested to explore whether rat-specific IgG_4 was associated with a reduction in the risk of symptoms of

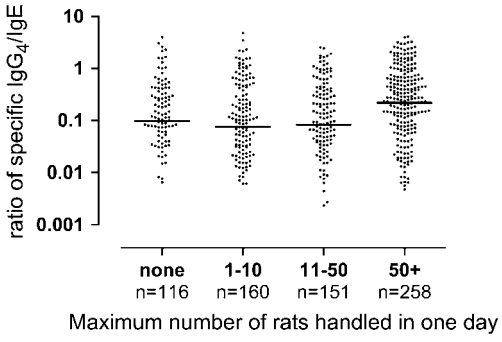

Figure 3. Ratios of IgG_4/IgE in the total population stratified by maximum number of rats handled in 1 d. *Horizontal lines* represent median ratios of IgG_4/IgE. $p < 0.01$.

TABLE 3. RESULTS FROM MULTIVARIATE LOGISTIC REGRESSION MODELING FOR WORK-RELATED CHEST SYMPTOMS

	Odds Ratios (95% CI)*	p Values
Specific IgE (only)	24.90 (9.98, 62.09)	< 0.001
Specific IgG_4 (only)	2.44 (0.95, 6.24)	0.07
IgE IgG_4 interaction term	0.23 (0.06, 0.89)	0.03
Specific IgE + IgG_4	13.91 (5.47, 35.36)	< 0.001
Atopy	2.15 (1.03, 4.47)	0.04
Exposure, maximum number of rats ever handled in one day		
0	1.00	0.10
1–10	4.19 (1.10, 15.95)	
11–50	1.89 (0.47, 7.70)	
50+	2.29 (0.60, 8.67)	

Definition of abbreviation: CI = confidence interval.
* Odds ratios adjusted for other factors in the table.

LAA. We found a twofold reduction in the risk of developing work-related chest symptoms in those who produced both rat-specific IgG_4 and IgE as compared with those producing only specific IgE (Table 3). This is, to the best of our knowledge, the first time that this interaction has been demonstrated.

Atopic individuals are at higher risk of LAA. In our population, there were fewer atopic employees among those in jobs with the highest exposure, a pattern that could have explained the reduced prevalence of rat-specific sensitization in this group. The pattern might, in theory, be the result of a higher proportion of nonatopic individuals entering high-exposure jobs (selection bias) or of a higher proportion of atopic individuals leaving them (survival bias). However, our extended analyses indicate that neither of these is a sufficient explanation for our findings. To investigate the potential role of a survivor effect in the current study, we restricted analysis to those employees with no more than 3 yr of exposure to rats, and observed the same pattern of high-dose attenuation. This was true also for individuals who had less than a single year of exposure to rats (data available on request). In addition, we found a reduction in the prevalence of IgE positive/asymptomatic individuals at high exposure (data available on request) where the observations cannot be explained by individuals leaving employment because of their work-related symptoms. Finally, we observed a similar exposure–response pattern in a 7-yr longitudinal study of laboratory animal workers with high rates of follow-up (3).

It is not likely that a selection bias of nonatopic individuals into heavily exposed jobs can explain the exposure–response pattern seen in our study. We demonstrated the same bell-shaped curve for sensitization in both atopic and nonatopic individuals. Although there were fewer susceptible (atopic) individuals in higher exposure jobs, the pattern of risk in the susceptible population is the same as that in the nonsusceptible group. Thus, we believe it is unlikely that either survival pressures or selection bias explains the patterns of exposure–response we have observed.

Inverse relationships between IgE sensitization and increased specific IgG and IgG_4 have been described as representing a "modified Th2 response," because both antibody classes require the Th2 cytokine interleukin 4 for their production. The term was first used to describe the apparent tolerance observed in children exposed to high levels of cat allergen who produced specific IgG and IgG_4 antibodies without IgE sensitization or asthma (1). A further study has confirmed cat-keeping to be associated with a modified Th2 response, which was not associated with an increased risk of either asthma or allergy. It was suggested

in that study that the shift, from IgE to IgG_4, resulted in less asthma and allergy due to lower IgE levels rather than because of a protective effect directly mediated by IgG_4 antibodies (11).

Ratios of specific IgE to IgG_4 change during specific immunotherapy. Specific serum levels of both IgE and IgG isotypes increase during the early phase of therapy, but the increase in specific IgG_4 is more pronounced and the ratio of specific IgG_4 to IgE increased by 10- to 100-fold, suggesting a protective role for IgG_4 (12). In our study, the ratios of specific IgG4 to IgE were increased in those with the highest exposure. Laboratory animal workers may, at very high exposures, be experiencing a natural form of immunotherapy; interestingly, this does not seem to be the case for other groups at risk of occupational asthma—for example, bakers (13) and detergent manufacturers (14). We propose that the differences arise because laboratory animal workers experience exposures not only through inhalation but also through an intradermal route after bites and scratches. The same mechanism may be relevant, as above, in the responses to domestic cat exposures—which likely include scratches—described by Platts Mills and colleagues (1), where high-dose tolerance was observed to cat allergen but not to house dust mite, for which instead there was a linear dose–response association. Consistent with our hypothesis is the state of naturally induced tolerance observed among healthy, hyperimmune subjects who have been frequently stung by bees. Beekeepers showed similar changes in their immune responses to those receiving therapeutic immunotherapy (15). Furthermore, repeated inhalation of cat peptides, as an experimental method of immunotherapy, was not—in contrast to an intradermal method—associated with the induction of hyporesponsiveness ("tolerance") in the skin or the lung (16).

A recent study in which the role of IgG_4 in LAA was investigated also reported that high exposure to rats was associated with a strong allergen-specific IgG_4 antibody response (8). These authors concluded that IgG_4 could not explain the absence of a dose response between allergen exposure and allergy in long-term exposed workers but was more likely to be a combined marker of exposure and susceptibility. Our findings, however, suggest that IgG antibodies themselves are protective. If this is so, then the question of a mechanism arises. Some studies have suggested that IgG antibodies could play a protective role by blocking leukocyte histamine release, inhibiting signal transduction and mediator release through the high-affinity IgE receptor (FcεRI) and IgG (FcγRIIB) receptors (17–20) and blocking allergen-induced IgE-dependent histamine release by basophils (21). Others have suggested that not only quantitative changes occur with IgG production but that the spectrum of the specificity of the IgG antibody is altered (22).

Our findings indicate that the relationship between allergen exposure and induction of allergy and asthma is complex. In particular, the attenuation of risk of IgE sensitization and asthma among those most heavily exposed is likely to be explained, at least in part, by a modified Th2 response. We have shown that IgG_4 production is driven by increasing allergen exposure and have demonstrated, for the first time, an interaction between IgE and IgG_4 whereby their coproduction is associated with a reduction in risk of IgE-associated, work-related respiratory disease. These findings require detailed, prospective study before their implications for the prevention of LAA can be interpreted.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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