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IgG4 antibodies against rodents in laboratory animal workers do not protect against allergic sensitization

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Abstract

Background: The modified Th2 response, defined as an IgG4 response in the absence of IgE, is suggested to protect against the development of allergic sensitization. However, studies suggesting this protective effect all had a cross-sectional design, making it impossible to study the development of both responses.
Aim of the study: We aimed to study the dynamics in IgG4 antibodies in relation to allergic sensitization in an occupational cohort of starting laboratory animal workers. Moreover, we studied the relation between exposure, antibody responses, atopy and self reported allergic symptoms.
Methods: A total of 110 starting animal workers were followed for 2 years. IgG4 antibodies against rats and mice were assessed. Workers were tested for allergic sensitization and exposure to animal allergens was estimated. Symptom status was assessed using questionnaires.
Results: Rat and mouse specific IgG4 antibodies were present before the development of allergy and did not significantly change over time. Allergic sensitization was related to exposure and atopic status but high levels of IgG4 showed no protective effect. In contrary, workers that developed mouse specific sensitization during follow up had higher levels of mouse specific IgG4. Symptoms were related to allergic sensitization and IgG4 levels did not influence that relationship.
Conclusions: IgG4 antibodies are present before IgE antibodies develop and IgG4 levels are stable over time. In our occupational cohort, the modified Th2 response had no protective effect on development of sensitization or allergic symptoms.

Allergic disease is associated with the presence of IgE antibodies. However, the majority of highly exposed individuals develop an IgG4 antibody response irrespective whether IgE is present (1–3). The B cell switch towards the production of IgG4 antibodies is dependent on the Th2 derived cytokine IL-4, while IL-10 can enhance the production of IgG4 by B cells (4, 5). This suggests that nonallergic individuals also have a Th2 response to allergens without production of IgE. The IgG4 response in the absence of allergic sensitization is referred to as a modified Th2 response (3). Atopic status may influence this response (6). Protective functions of modified Th2 responses for the development of sensitization are suggested (3, 7, 8). However, other studies did not find this protective effect of IgG4 on the occurrence of allergy (1, 9, 10). All these studies had a cross-sectional design, making it difficult to determine if IgG4 or IgE antibodies develop first and if IgG4 antibodies can prevent the development of IgE antibodies or ameliorate allergic symptoms (11).

We studied the development IgG4 and IgE antibody responses in our cohort of laboratory animal workers (12, 13). In addition we studied the dynamics in IgG4 responses over time and possible relationships between IgG4 antibodies, exposure, atopy and the development of IgE and allergic symptoms.

Methods

Subjects

Starting animal handlers with no more than 18 months experience with animal work in their present job and without allergic sensitization to the animals they were handling were

included in the study. A total of 110 laboratory animal workers were followed for 2 years. Study visits took place at the start of the study (visit A), after 4 months (visit B), after 12 months (visit C) and at the end of follow up (visit D). The Medical Ethical Board of the Academic Medical Center of the University of Amsterdam approved the study. All participants gave informed consent and completed a questionnaire on their health status, allergic symptom and animal contact hours per month before each visit. Baseline characteristics of the subjects are in Table 1.

Exposure

Personalized exposure to rat and mouse urinary allergens [RUA and MUA, in ng equivalent (eq)/m^3 (12)] was calculated by multiplying self reported contact hour per month by exposure measured in the specific facility with personal active sampling as described (12). The mean exposure per month of the total follow up was used. To correct for the possible influence of sensitization on exposure for the participants developing sensitization, their mean exposure per month was calculated until the moment sensitization was detected. Animal workers were divided into quintiles based on their exposure. Analyses were performed on these quintiles as well as on continuous measures.

Sensitization, IgE and IgG4

Rat or mouse specific allergic sensitization was based on skin prick test results and/or a positive IgE test (>0.35 IU/ml) as described before (12).

During the 2 years of follow up, blood was taken at each visit. Serum samples were stored at −20°C until analysis. Rat and mouse specific serum IgE and IgG4 levels were measured with radio-allergosorbent testing [RAST (13, 14)]. IgE and IgG4 levels were measured against freeze dried rat or mouse urine extract. IgE levels were expressed as International Units per milliliter (IU/ml) while IgG4 results were read from a standard curve with a mouse/human chimeric monoclonal IgG4 antibody to Der p 1 and expressed in nanograms per milliliter (ng/ml). The detection limit was 0.15 IU/ml for IgE and 10 ng/ml for IgG4. Values below the limit of detection were allotted a value of half the detection limit. IgG4 levels were also measured in sera from 25 controls without occupational exposure to rodents. Atopic status was based on IgE RAST tests to common allergens (house dust mite, grass pollen, birch pollen, cat, dog) (12, 13).

Table 1 Characteristics of the cohort

Characteristics	*n* = 110
Age (mean, range)	26 (18–43)
Gender (male/female)	50/60
Smoking (*n*, %)	18 (16%)
Atopy (*n*, %)	43 (39%)
Baseline total IgE (IU/ml, GM, GSD)	37 (3,5)
Developed sensitization (*n*, %)	22 (20%)
Self reported allergic symptoms at visits B, C and/or D (*n*, %)	25 (23%)
Baseline IgG4 against RUA at baseline (ng/ml, GM, GSD)	480 (4,2)
Baseline IgG4 against MUA at baseline (ng/ml, GM, GSD)	121 (2,2)
Time animal work in current job at inclusion in the study (months, mean, range)	5 (0–18)
Time animal work in previous job or during education (months, mean, range)	5 (0–48)
Reported to handle rats (*n*, %)	53 (48%)
Reported to handle mice (*n*, %)	75 (68%)

Statistical analysis

Statistical analysis was done with SPSS version 16 Statistics (Chicago, IL, USA). IgE, IgG4 and exposure results were evaluated in terms of their log-value and results are expressed as geometric mean and geometric standard deviation (GSD).

Correlations of continuous measures were analyzed with Spearman's correlation, while data in quintiles were analyzed with the Cuzicks test for trends (15). Differences between groups were tested with Student's *t*-test. Relations between symptoms and other variables were tested with (multiple) logistic regression analysis. *P*-values below 0.05 were considered statistically significant.

Results

Study population

Characteristics of this cohort are in Table 1. Of the 110 participants analyzed, 2 were lost to follow up after visit B and 4 were lost after visit C. More females than males participated in the study and the mean age of the participants was 26 years. Atopy was found in 39% of the participants and 16% were current smokers. The majority of the participants worked with mice and 16% handled both rats and mice.

Exposure

At inclusion, the mean duration of previous animal contact during education, in previous jobs and in the present job was 10 months (range: 0–61 months, Table 1).

Self reported animal contact hours during follow up ranged from 1 to 173 h per month. Exposure was assessed in 29 different working zones. These measurements showed that all workers were exposed to both rat and mouse allergens, although their exposure was highest for the species they handled. The highest RUA exposure in our cohort was 12 226 ng eq./m^3*h/month, while the highest MUA exposure was 78 297 ng eq./m^3*h/month.

Sensitization and IgE

During 2 years follow up, 22 people developed a new sensitization against rat ($n = 18$) and/or mouse allergens ($n = 12$) as detected with skin prick test ($n = 21$) and/or RAST

Table 2 Sensitization and IgG4 in quintiles of exposure

Quintiles	1	2	3	4	5	*P**
Animal contact[1]	< 7	7–16	16–29	29–65	> 65	
Number of workers	22	22	22	22	22	
Sensitized against lab. animals (*n*, %)	1 (5%)	5 (23%)	2 (9%)	8 (36%)	6 (27%)	**0.029**
Sensitized against rats (*n*, %)	0 (0%0	4 (18%)	2 (9%)	6 (27%)	6 (27%)	**0.011**
Sensitized against mice (*n*, %)	1 (5%)	2 (9%)	1 (5%)	5 (23%)	3 (14%)	0.169
RUA exposure[2]	< 5	5–42	42–400	400–1700	> 1700	
Number of workers	22	22	22	21	21	
Sensitized against rats (n, %)	2 (9%)	1 (5%)	4 (18%)	3 (14%)	8 (38%)	**0.009**
rIgG4[3] (GM, GSD)	372 (4,4)	447 (5,4)	691 (3,1)	646 (3,7)	302 (3,0)	0.890
MUA exposure[2]	< 25	25–110	110–600	600–1500	> 1500	
Number of workers	22	22	22	22	21	
Sensitized against mice (*n*, %)	1 (5%)	2 (9%)	5 (23%)	2 (9%)	2 (9%)	0.628
mIgG4[3] (GM, GSD)	87 (2,5)	112 (1,8)	178 (2,5)	100 (2,3)	129 (3,0)	0.880

[1]h/month, [2]ng eq./m[3]*h/month, [3]visit D, ng/ml.

**P*-value from Cuzick test for trends

GM, geometric mean; GSD, geometric standard deviation. Bold text is *P*-value <0.05

(n = 16). We reported before that development of sensitization was related to atopic status (12). Increased number of animal contact hours per month resulted in increased percentage of sensitization (rho = 0.202, P = 0.036). Moreover, development of sensitization to rats was related to level of rat allergen exposure per month (continuous measure: rho = 0.241, P = 0.011; in quintiles see Table 2). Rat specific IgE (rIgE) levels at visit C and D correlated with exposure to rat allergens during follow up (for visit D: r = 0.248, P = 0.01, rest not shown). No such relations were found for mouse specific IgE (mIgE) and exposure.

IgG4

At visit A, rat specific IgG4 levels (rIgG4) and mouse specific IgG4 levels (mIgG4) in sera of animal workers were significantly higher than in sera from controls without occupational exposure (Fig. 1). Surprisingly, during follow up with ongoing exposure, no significant changes in IgG4 antibody levels of the animal workers were detected (Fig. 2). In workers, mIgG4 levels at visit A correlated with self reported total duration of previous animal contact (rho = 0.303, P = 0.01) but rIgG4 levels did not. No relation between IgG4 antibody levels and exposure during the study expressed as continuous variable or in quintiles (Table 2) was found in animal workers. Also no correlations were found between self reported animal contact hours per month and IgG4 levels. IgG4 levels for atopics and nonatopics were similar (Fig. 3).

We found no significant relationship between rIgE and rIgG4 antibody levels. However, we found a weak trend for a positive relationship for mIgG4 and mIgE (rho = 0.177, P = 0.075). Also workers developing mouse specific sensitization during follow up had significantly higher levels of mIgG4 at all visits, even before they developed their sensitization (all visits: P < 0.01, Fig. 3B). This was not found for rat (Fig. 3 A). Self reported allergic symptoms during animal work were related to a positive skin prick test, rat specific IgE and mouse specific IgE levels but not to IgG4 levels. In a multiple logistic regression model sensitization was the best predictor for symptoms (data not shown). Specific IgG4 levels did not modify this relationship nor was IgG4 related to a reduction of allergic symptoms in newly sensitized workers.

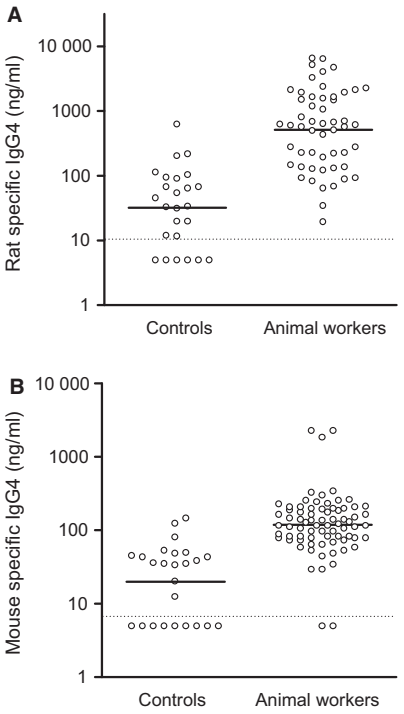

Figure 1 IgG4 in animal workers and controls. Levels of rat (A) and mouse (B) specific IgG4 in controls and laboratory animal workers (visit A). The dotted line is the limit of detection. Controls had significantly lower IgG4 levels compared to animal workers at baseline (P < 0.001).

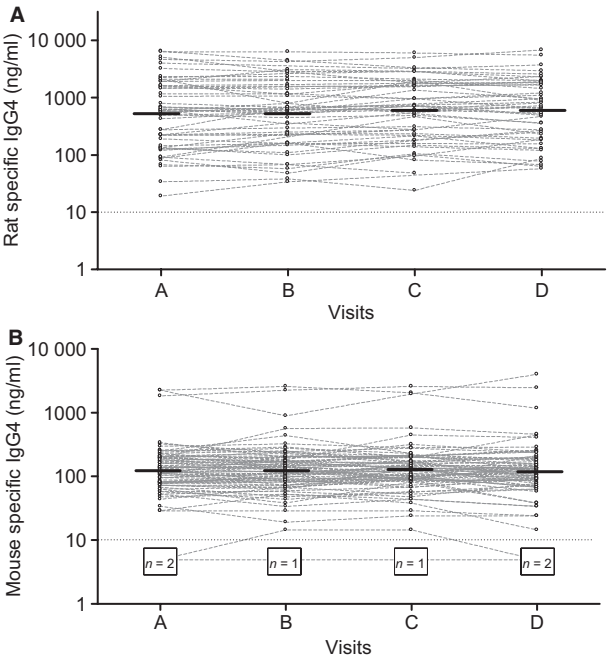

Figure 2 IgG4 during follow up. Levels of rat and mouse specific IgG4 levels in rat handling (A, $n = 53$)) and mouse handling (B, $n = 75$) laboratory animal workers during follow up. Boxed are the numbers of samples below the limit of detection (dotted line). No specific changes occurred during follow up in both rat and mouse specific IgG4.

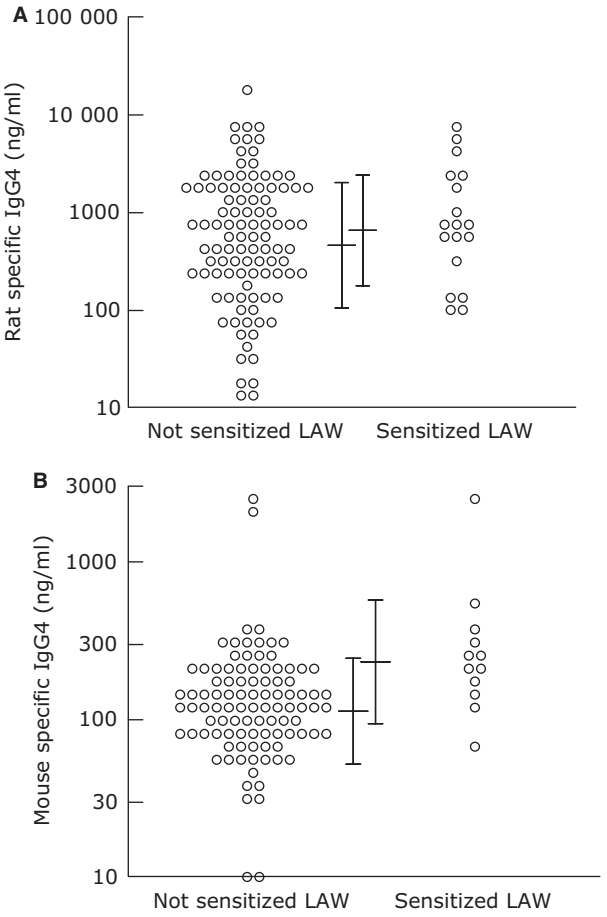

Figure 3 Sensitization and IgG4 levels at visit A. Animal specific IgG4 levels at visit A. (A) Rat sensitized workers ($n = 18$) had no significantly different levels of rat specific IgG4 before the development of sensitization (visit A) or at all other visits (data not shown). (B) Workers that would become sensitized to mice ($n = 12$) had slightly but significantly higher levels of mouse specific IgG4 at the start of the study and during follow up (data not shown). Laboratory Animal Worker (LAW).

Discussion

The present study shows the dynamics in IgG4 antibodies during prolonged follow up of occupationally exposed starting laboratory animal workers and its relation to allergic sensitization and exposure. We tested the hypothesis of a protective effect of the modified Th2 response on sensitization. Workers in our cohort with high specific IgG4 were not protected against the development of IgE.

The sample size of 110 animal workers in this study is relatively small. Although we observed new sensitization in as much as 20% of our population, the number of sensitized workers remains small. To minimize the effect of small groups in the Cruzick analysis in quintiles, we repeated all tests in quartiles and tertiles (data not shown) as well as in continuous measures. These additional tests gave comparable results. However, we can not exclude that the low sample size obscured small effects, either protective or enhancing, of IgG4 on allergic sensitization.

Already at the start of the study rIgG4 and mIgG4 levels in workers were high compared to unexposed controls. IgG4 antibody responses normally develop in months rater than weeks (16). Animal contact before the start of the study, which was possibly underestimatedion of this contact by the questionnaires, may have contributed to the high IgG4 levels at visit A. This is supported by the relationship between mIgG4 levels at visit A and self reported previous exposure, confirming an exposure.

During follow up, IgG4 levels did not change significantly. Previously we showed comparable stable IgG4 levels in a group of workers occupationally exposed to bovine and porcine serum proteins (1). Relations between cumulative exposure and IgG4 levels in studies of animal workers were reported before in cross-sectional studies (2, 10). Probably IgG4 responses occur within a few months after first exposure and we may have missed this response because this occurred during animal contact before inclusion to the study. Another possible explanation for the absence of an exposure-response relationship for IgG4 during the study may be that the range of exposure in the present study was too small to find differences in IgG4 levels between differentially exposed workers. In the previously mentioned paper on bovine and porcine specific IgG4 (1), a greater range in exposure was present. The follow up of 2 years was possibly also too short to see dynamics in the IgG4 response. However, the mean

exposure duration in a previous cross-sectional study in mouse workers (2) was also 3 years.

Exposure–response relationships that were found for IgE, sensitization and IgG4 were different for rat than for mouse. However, the measured range of exposure to rat and mouse urinary allergens were comparable. Although major allergens from rats and mice are urinary proteins and both are lipocalins (14), their allergenetic potency to induce IgE and IgG4 seems different. Especially for IgE, we saw more sensitization for rat allergens. Differences in potency and the ability to induce an IgG4 response was also found other allergens like house dust mite and cat (3, 16) and may influence found relationships.

The modified Th2 response is often suggested to protect against the development of sensitization (3, 7, 11). In our cohort, we found no protective effect of IgG4 antibodies on allergic sensitization as detected by skin prick test or IgE RAST. Previous findings of a protective effect of the modified Th2 response in laboratory animal workers (7, 8) may have been influenced by their cross-sectional design. In a cross-sectional study, the development of responses can not be followed. In addition, the healthy workers effect is difficult to exclude. We showed that IgG4 responses develop earlier than IgE responses in all workers that became sensitized. In the study from Jeal et al. (7), who claimed a protective effect of IgG4, ratios of IgG4 to IgE were studied. We believe this may not be appropriate as sensitization leads to increased IgE levels and therefore influences the IgG4:IgE ratio *per se*. Our design enabled us to purely study the protective effect of IgG4 on new sensitizations as we measured IgG4 levels prior to the development of sensitization. In this design high levels of IgG4 prior to sensitization were not protective in our cohort. For sensitization to mice, we even found that high levels of mIgG4 at visit A (and all other visits) were positively associated with development of IgE during follow up. This may suggest that high IgG4 is even a risk factor for sensitization. Other (cross-sectional) studies also have suggested this before (8, 10). Aalberse et al. (6) described the possibility that IgE antibodies develop via the B-cell switch from γ4 to ε, especially in nonatopics. The high mIgG4 and rIgG4 levels before the development of IgE antibodies in our study is in agreement with this hypothesis and this suggestion may even be true for atopics. The group of nonatopic sensitized workers in our cohort was too small to analyze this group separately.

Another role that was suggested for IgG4 is that it can act as a blocking antibody and prevent or reduce allergic symptoms (1, 7, 11, 16). We found no evidence for a protective role for IgG4 on manifestation of allergic symptoms in our cohort. However, the levels of IgG4 found in sera of our animal workers are lower than the levels found before (1) and we had less sensitized workers to study this protective effect. It is possible that higher levels of IgG4 induced by prolonged exposure may result in a blocking effect on symptoms.

In conclusion, our longitudinal study in laboratory animal workers showed that IgG4 antibody responses develop prior to IgE antibody responses. These IgG4 responses were stable over the time studied and were not related to measured exposure. Allergic sensitization was positively associated with exposure and atopy. IgG4 responses did not protect against allergic sensitization or against manifestation of allergic symptoms. These findings are compatible with the hypothesis that IgE antibody responses in these occupationally exposed individuals may arise from a class switch of IgG4 producing B-cells.

Author contribution

E.J.M. Krop acquired data, performed the laboratory and statistic analyses and wrote the manuscript, G. Doekes performed the exposure assessment and was involved the writing of the manuscript, D. Heederik was involved in the exposure assessment and the statistical analyses, R. Aalberse was involved in the IgG4 and IgE testing and design of the study and J. van der Zee designed the study, was involved in the statistical analyses and the writing of the manuscript.

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Conflict of interest

There are no known conflicts of interest for the authors.

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