

J. Uitti · H. Nordman · L. Halmepuro · J. Savolainen

IgG₄ response to fur animal allergens among fur workers

Received: 10 November 2003 / Accepted: 25 June 2004 / Published online: 9 December 2004
© Springer-Verlag 2004

Abstract Objective: The aim of this study was to determine whether IgG₄ antibodies to allergens in urine extracts from fur animals associated with positive prick tests to the same allergens and with the occurrence of respiratory symptoms among fur workers, especially among highly exposed fur farmers. **Methods:** Among the fur workers and among their referents, IgG₄ antibodies to mink and silver fox urine were analysed in three groups; all workers with a positive skin prick test to any fur animal allergen ($n=50$), all workers who had reported shortness of breath or rhinitis or eye symptoms ($n=159$), and to a random sample of asymptomatic persons ($n=178$). In the two last groups none of the workers had a positive skin test to any fur animal allergen. **Results:** The fur farmers had higher level of IgG₄ values than other groups and also had positive IgG₄ antibodies to urine extract more frequently than the other groups. Among the exposed subjects, there was a distinct overlapping of a positive skin prick test to fur urine allergens and positive IgG₄ antibodies to responding allergens. Among the fur farmers the IgG₄ levels were associated with symptoms. **Conclusions:** IgG₄ antibodies were shown to be a good indicator of exposure. Because of an overlapping of positive skin prick tests and IgG₄ response to the same allergens, and an association between symptoms and IgG₄ response, it is

recommended that the potential role of IgG₄ antibodies as an indicator of alternative sensitisation should be further examined in prospective studies.

Keywords Respiratory symptoms · Exposure to fur animal allergens · Skin prick tests · IgE response · IgG₄ antibodies

Introduction

Immunoglobulin G (IgG) antibodies in the form of antigen-specific IgG₄ reflect exposure to the antigens in question. Several arguments have been presented for a pathogenic role of IgG₄ antibodies (Gwynn et al. 1978, 1979; Pepys 1979). In occupational environments a history indicating an immediate-type of allergy without demonstrable specific IgE has been observed among bakers (Kraut et al. 1992). In studies of allergy caused by phthalic anhydrides, an association has been suspected between IgG₄ antibodies and hypersensitivity symptoms (Nielsen et al. 1988; Nordman et al. 1987) or phthalic anhydride allergy (Pakarinen et al. 2002), but also studies on the same topic have been published that have not shown any association (Yokota et al. 1997).

In one cross-sectional study, we found specific IgE sensitisation to fur animal allergens among fur farmers and fur garment workers (Uitti et al. 1997). The number of IgE sensitised workers was surprisingly small, so the selection was confirmed with a supplementary questionnaire sent to former workers. In spite of this selection, an excess of upper respiratory and eye symptoms was observed among fur workers, and the excess was explained by irritation from fur dust or by unknown hypersensitivity mechanisms. The aim of this study was to determine whether specific IgG₄ antibodies to allergens in fur animal urine were associated with positive skin prick tests to the same fur animal allergens and with respiratory symptoms among fur workers, especially among highly exposed fur farmers.

J. Uitti (✉)
Tampere Regional Institute of Occupational Health,
Clinic of Occupational Medicine, Tampere University Hospital,
486, 33101 Tampere, Finland
E-mail: Jukka.Uitti@ttl.fi
Tel.: +358-0304748655
Fax: +358-3-0304748699

H. Nordman · L. Halmepuro
Finnish Institute of Occupational Health,
Helsinki, Finland

J. Savolainen
Department of Clinical Allergology, University of Turku,
Turku, Finland

Materials and methods

Subjects

In our study, fur farmers ($n=188$) were compared with workers ($n=181$) from a plastic factory (Uitti et al. 1997). The fur garment workers ($n=175$) had unexposed clerical referents ($n=118$). The study comprised a self-administered questionnaire, lung function tests, and skin prick tests to common environmental allergens, domestic animals, fur and urinary allergens of fur animals.

IgG₄ antibodies were analysed for the fur workers and their referents in three groups. The first group comprised all those with a positive prick skin test to any fur animal allergen ($n=50$), the second and third groups were selected from persons in study populations with no positive skin tests to any fur animal allergen. These groups comprised all those who had reported shortness of breath or rhinitis or eye symptoms ($n=159$) in questionnaires and a random sample of asymptomatic persons ($n=178$). In the analysis of the results the outcome variables were compared between the different exposure groups, but, in addition, the results were analysed only for the exposed groups, the fur farmers and fur garment workers. Among them there were 28 persons with any positive skin prick test to fur allergen, 99 symptomatic persons, and a responding sample of 111 asymptomatic workers.

Allergen extracts

The urine extracts of mink (*Mustela vison*), silver fox (*Vulpes vulpes*) and blue fox (*Alopex lagopus*) were prepared as described earlier (Savolainen et al. 1997; Uitti et al. 1997). The urine allergens had earlier been found to contain more protein, including allergens, than fur extracts did. They were thus selected for the analysis of IgG₄ antibodies. Urine specimens were obtained also from mink with plasmacytosis (aleuthian disease), a widespread viral disease causing marked proteinuria among mink. The following four extracts were prepared for this study: urine of mink with plasmacytosis (MU+), urine of healthy mink (MU-), urine of silver fox (SFU), urine of blue fox (BFU). The earlier studies (Savolainen et al. 1997; Uitti et al. 1997) indicated that the most potent urine extracts allergenically were mink urine with plasmacytosis (MU+) and silver fox urine (SFU), which were therefore used in the analyses in this study.

Antibodies

Mono-clonal anti-human IgG₄ antibodies (clone GB7B) were purchased from Oxoid Ltd (UK). The antibodies belonged to the mouse IgG₁ subclass. Sheep antimouse IgG conjugate labelled with alkaline phosphatase was obtained from Sigma Chemical Co. (USA).

Other reagents

Bovine serum albumin (BSA) and para-nitrophenyl phosphate (pnPP) substrate tablets (5 mg) were purchased from Sigma. Phosphate-buffered saline (PBS), pH 7.4, was used as the coating buffer, whereas, the dilution buffer was PBS-0.5% Tween 20-1 mmol/l MgCl₂·6H₂O-0.1% BSA for the test sera and antisera, the substrate buffer was 10% diethanolamine, pH 9.8, the stop solution was 3 mmol/l NaOH, and the washing buffer was PBS-0.05% Tween 20.

IgG₄ enzyme immunoassay

The enzyme linked immunosorbent assay (ELISA) was performed essentially as described by Tiikkainen and Klockars (1989). Flat-bottomed polystyrene microtitre plates (Microstrips; Eflab Ltd, Finland) were coated with 125 µl of the urine extracts. After serum incubation the bound IgG₄ antibodies were detected with mouse monoclonal anti-human IgG₄ antibodies and alkaline-phosphatase-labelled sheep anti-mouse IgG and chromogenic substrate. The measured absorbances were changed to arbitrary units by comparing the absorbances of test sera with the sera of the unexposed controls, and a ratio of 2 was considered positive (2 SD for the controls).

Skin prick tests and specific immunoglobulin E (IgE) antibodies

The detection of IgE antibodies has been described earlier (Savolainen et al. 1997). The skin prick tests were performed according to EAACI position paper.

Statistical methods

Standard descriptive statistics were used to represent the responses. Cross-tabulations with Chi-square and Fisher's exact tests were used to determine if the relationships between the categorized variables were statistically significant. Mann-Whitney *U*-test was used to compare the difference between the groups in values of IgG₄ titres which were not normally distributed. Association of symptoms with IgG₄ antibody titres were considered with a logistic regression model which included age, smoking and atopy as covariates in addition to the IgG₄ antibody titre.

Results

Exposure and sensitisation to fur allergens

The values of IgG₄ antibodies to MU+ or to SFU were highest among fur farmers, overall higher in exposed groups than in referents (Table 1). The employment time

of the fur farmers did not correlate with positive IgG₄ to MU+ or to SFU. When combined, the exposed groups had higher occurrence of positive IgG₄ antibodies to mink urine (MU+) and to silver fox urine (SFU) than the unexposed groups ($P < 0.0001$): Among the exposed workers 16% had positive IgG₄ antibodies to mink urine (MU+) and 14% to silver fox urine (SFU), while none of the unexposed controls showed a positive MU+ and 2% had positive antibodies to SFU.

Among the exposed fur workers ($n = 238$) there were nine subjects with a positive skin prick test to MU+, 56% (5/9) of whom had positive IgG₄ to MU+. Among those with a positive skin prick test to SFU, 42% (5/12) had positive IgG₄, respectively. Of the subjects with a negative skin prick test to MU+ or SFU, 14% ($P = 0.006$) and 13%, respectively ($P = 0.017$) had positive IgG₄ to the same antigens. Three fur farmers with respiratory symptoms and specific IgE antibodies (RAST) to fur animal allergens also had positive IgG₄ values.

Symptoms

Among the fur workers, especially among farmers those with symptoms had higher levels of IgG₄ titres to MU+ and SFU than unsymptomatic had (Table 2). In the

logistic regression analysis adjusted with smoking and age symptoms of the fur farmers were associated with IgG₄ levels to MU+ ($P = 0.0027$) or SFU ($P = 0.0014$) separately. By adding other confounders as atopy or earlier allergic diseases to the model both IgG₄ antibodies remained as significant explaining factors. In addition, smoking was a significant explaining factor in the model. In fur garment workers none of the factors and in the combined group (fur workers) only smoking was significantly associated with symptoms.

There were six IgG₄ positive persons (MU+ or SFU), five of whom had rhinitis symptoms but only two of whom had a positive skin test to the same allergen. After further clinical examinations, including nasal provocation tests, four cases of rhinitis were diagnosed as occupational disease. All three of the fur farmers who were RAST positive and IgG₄ positive to fur allergens had contracted respiratory symptoms. In addition, four of the 15 symptomatic fur farmers with negative skin tests to fur allergens and positive IgG₄ had shortness of breath.

Discussion

In our study, the IgG₄ antibodies to mink and silver fox urine of the fur farmers seemed to be a proper indicator

Table 1 IgG₄ titres among fur farmers, their referents, fur garment workers (*fur gw.*) and their referents

	MU				SFU			
	Mean	Median	Range	P^*	Mean	Median	Range	P^*
Fur farmers ($n = 117^a$)	4.20	1.20	0.9–21.6	< 0.0001	2.19	1.10	0.8–17.3	0.0003
Referents ($n = 87$)	1.12	1.10	0.9–1.6		1.05	1.00	0.8–2.0	
Fur gw. ($n = 121^a$)	1.23	1.10	0.9–9.3	0.47	1.08	1.00	0.9–3.3	0.0063
Referents ($n = 61$)	1.16	1.10	0.9–1.7		1.14	0.9	0.8–9.0	
Fur workers ($n = 238$)	2.69	1.15	0.9–21.6	0.0012	1.62	1.00	0.8–17.3	0.0001
Referents ($n = 148$)	1.13	1.10	0.9–1.7		1.09	1.00	0.8–9.0	

* P , difference between exposed and unexposed group, Mann-Whitney U -test

^a Difference of both IgG₄ titres between fur farmers and fur garment workers was significant $P < 0.0001$

Table 2 IgG₄ titres among symptomatic (*sympt.*) and unsymptomatic (*unsympt.*) fur farmers, fur garment workers (*fur g. w.*) among all fur workers and their referents

	MU				SFU			
	Mean	Median	Range	P^*	Mean	Median	Range	P^*
<i>Fur farmers</i>								
Symptomatic ($n = 42$)	6.25	1.40	1.0–21.1	0.0069	3.13	1.20	0.9–17.3	0.08
Unsymptomatic ($n = 75$)	3.05	1.20	0.9–21.6		1.66	1.10	0.8–10.3	
<i>Fur g.w.</i>								
Symptomatic ($n = 72$)	1.26	1.10	0.9–9.3	0.0058	1.06	1.00	0.9–3.3	0.0002
Unsymptomatic ($n = 49$)	1.19	1.10	1.0–1.7		1.11	1.00	0.9–2.0	
<i>Fur workers</i>								
Symptomatic ($n = 114$)	3.10	1.10	0.9–21.1	0.3281	1.82	1.00	0.9–17.3	0.0367
Unsymptomatic ($n = 124$)	2.31	1.20	0.9–21.6		1.44	1.10	0.8–10.3	
<i>Referents</i>								
symptomatic ($n = 80$)	1.16	1.10	0.9–1.7	0.1435	1.08	0.90	0.8–9.0	0.0002
Unsymptomatic ($n = 68$)	1.11	1.10	0.9–1.5		1.09	1.05	0.9–2.4	

* P , difference between exposed and unexposed group, Mann-Whitney U -test

of exposure. In addition, these antibodies reflected positive skin prick tests to the same allergens and were associated with symptoms among the exposed workers, especially in fur farmers.

The IgG₄ antibodies were analysed in samples from both exposed and unexposed workers. We considered it necessary to use the sera of controls because of the cross-reactivity between IgE antibodies to fur and domestic animal allergens (Savolainen et al. 1997). This cross-reactivity also explains the positive titres of IgG₄ in the unexposed group. In our study the employment time did not correlate with the positivity of the IgG₄ antibodies. The complex working environment of fur work, consisting of peak exposures and varying outside working conditions, makes it impossible to assess differences in exposure levels (Schimberg et al. 1992; Uitti et al. 1997).

The IgG₄ antibodies correlated with the positive skin prick tests to fur allergens. On the level of the total allergen molecule, responses to IgE and IgG₄ antibodies responses have been found to be associated (Lucas 1992; Yokota et al. 1998). In laboratory studies many proteins in wheat flour can bind IgG₄ and IgE, but also a reasonable similarity of major allergens has been detected by the IgG₄-serum pool and IgE-positive sera (Houba et al. 1996). The fur animal allergens were not defined in our study (Savolainen et al. 1997); therefore, it was not possible to investigate responses to separate epitopes. In every case, our findings resembled those of bakers exposed to wheat flour, for which the presence of IgG₄ and IgE antibodies may be interrelated (Matsumura et al. 1994; Tiikkainen and Klockars 1989).

The symptoms of fur farmers, mostly exposed to mink or silver fox urine, were associated with positive IgG₄ antibodies to these allergens. Additional clinical examinations revealed four cases with occupational rhinitis who had positive IgG₄ antibodies to urine extract from either mink or silver fox (Uitti et al. 1997). Despite of the selection of study population our findings suggest that IgG₄ was not only associated with exposure to antigens or with IgE antibodies to the same offending allergens, but also it may have enhanced the symptoms of the highly exposed group. The same kind association has been found in other work environments (Hollander et al. 1994; Kraut et al. 1992; Yokota et al. 1998). Contradictory results have also been published for Finnish bakers (Tiikkainen and Klockars 1989) and Dutch potato workers (Zock et al. 1996).

Follow-up studies, especially among newcomers or persons who have changed jobs, would help clarify these complex interrelationships between specific IgE sensitisation (skin prick tests) and specific IgG₄ antibodies, and symptoms.

Acknowledgements This study was supported by the Finnish Work Environment Fund and the Medical Research Foundation of the

Tampere University Hospital. We thank Kirsi Saarenpää for tasks in laboratory analysis.

References

- Gwynn GM, Smith JM, Leon GL, Stanworth DR (1978) Role of IgG4 subclass in childhood allergy. *Lancet* 1:910-911
- Gwynn GM, Smith JM, Leon GL, Stanworth DR (1979) IgE and IgG4 subclass in atopic families. *Clin Allergy* 9:119-123
- Hollander A, Heederik D, Kauffman H (1994) Acute respiratory effects in the potato processing industry due to bioaerosol exposure. *Occup Environ Med* 51:73-78
- Houba R, Van Run P, Heederik D, Doekes G (1996) Wheat antigen exposure assessment for epidemiological studies in bakeries using personal dust sampling and inhibition ELISA. *Clin Exp Allergy* 26:154-163
- Kraut A, Peng Z, Becker AB, Warren CPW (1992) Christmas candy maker's asthma; IgG4-mediated pectin allergy. *Chest* 102:1605-1607
- Lucas AH (1992) IgG subclass-restricted immune responses to allergens. *Springer Semin Immunopathol* 12:385-400
- Matsumura Y, Niituma T, Ito H (1994) A study of factors contributing to bakers' allergy symptoms. *Arerugi* 43:625-633
- Nielsen J, Welinder H, Schutz A, Skerfving S (1988) Specific serum antibodies against phthalic anhydride in occupationally exposed subjects. *J Allergy Clin Immunol* 82:126-133
- Nordman H, Keskinen H, Tiikkainen U, Tupasela O, Ahlberg R (1987) Experience of specific antibodies in the diagnostics of phthalic anhydride allergy. In: 22nd International Congress on Occupational Health, Sydney, Australia
- Pakarinen M, Koivuluhta M, Kalkkinen N, Keskinen H, Nordman H, Estlander T, Tupasela O, Jolanki R, Lauerman AI, Pfäffli P, Alenius H (2002) Phthalic anhydride allergy: development and characterization of optimized hapten-carrier conjugates for improved diagnosis. *Allergy* 57:894-899
- Pepys J, Parish W, Stenius-Aarniala B, Wide L (1979) Clinical correlations between long-term (IgG S-TS) anaphylactic antibodies in atopic and non-atopic subjects with respiratory allergic disease. *Clin Allergy* 9:645-658
- Savolainen J, Uitti J, Halmepuro L, Nordman H (1997) IgE response to fur animal allergens and domestic animal allergens in fur farmers and fur garment workers. *Clin Exp Allergy* 27:501-509
- Schimberg RW, Uitti J, Kotimaa M, Sarantila R (1992) Airborne particulate matter, fungi, bacteria and endotoxins in fur farming. *Staub Reinhaltung der Luft* 52:457-460
- Tiikkainen U, Klockars M (1989) Determination of IgG subclass antibodies against wheat flour antigens by an ELISA technique. *Allergy* 44:419-426
- Uitti J, Nordman H, Halmepuro L, Savolainen J (1997) Respiratory symptoms, pulmonary function and allergy to fur animals among fur farmers and fur garment workers. *Scand J Work Environ Health* 23:428-434
- Yokota K, Johyama Y, Yamaguchi K, Fujiki Y, Takeshita T, Morimoto K (1997) Specific antibodies against methyltetrahydrophthalic anhydride and risk factors for sensitization in occupationally exposed subjects. *Scand J Work Environ Health* 23:214-220
- Yokota K, Yamaguchi K, Takeshita T, Morimoto K (1998) The significance of specific IgG₄ antibodies to methyltetrahydrophthalic anhydride in occupationally exposed subjects. *Clin Exp Allergy* 28:694-701
- Zock JP, Doekes G, Heederik D, Van Zuylen M, Welaard P (1996) Airborne dust antigen exposure and specific IgG response in the potato processing industry. *Clin Exp Allergy* 26:542-548