

Airborne dust antigen exposure and specific IgG response in the potato processing industry

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Summary

Background High prevalences of work-related respiratory symptoms in relation to organic dust exposure have been reported in the potato processing industry, but the responsible effect mechanisms are not known.

Objective To study the possible role of a type III allergy in aetiology.

Methods Specific immunoglobulin G (IgG) and IgG₄ subclass antibodies against occupational airborne antigens were determined in sera from 131 potato processing workers and 36 non-exposed controls. Personal exposure to airborne antigens was measured, and a preliminary biochemical characterization was carried out.

Results Specific IgG was detectable in almost all sera, but levels were significantly ($P < 0.01$) higher in potato processing workers compared with controls. Specific IgG₄ was detectable in half of the workers' sera, but in none of the control sera. The antigens involved appeared to be heat-labile potato proteins. Antibody levels increased during the processing campaign in most workers, and this increase was dependent on the level of antigen exposure. Both the difference in IgG titres between the occupationally exposed group and the non-exposed group, and the exposure-related increase in specific IgG titres seemed to be mainly due to specific antibodies of the IgG₄ subclass. Specific antibodies showed a non-significant tendency to lower levels in workers with work-related respiratory symptoms.

Conclusion Occupational respiratory exposure in the potato processing industry leads to a strong humoral immune response, most pronounced for IgG₄ subclass antibodies. Type III allergy is, however, unlikely to play a predominant role in the aetiology of respiratory effects.

Keywords: IgG, IgG₄, potato antigens, type III allergy

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Introduction

Processing of agricultural products involves occupational exposure to several constituents of organic dust [1,2], a well-known risk factor for several respiratory diseases [3]. In the potato processing industry high levels of dust, micro-organisms, endotoxin and antigens can be encountered, accompanied by high prevalences of work-related

respiratory symptoms and peak flow patterns suggestive of reversible airway obstruction [4–6]. High levels of specific immunoglobulin G₄ (IgG₄) antibodies to airborne dust antigens were observed, whereas specific IgE antibodies could not be demonstrated and other IgG subclass antibodies were not measured. It was suggested that the observed work-related symptoms might be caused by an exceptionally high occupational endotoxin exposure, with full-shift average levels up to 30 000 Endotoxin Units per m³ [6]. An alternative possibility, that symptoms might be due to a type III allergic reaction could, however, not be excluded.

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The presence of specific IgG antibodies against occupational antigens has been described for several occupational environments with organic dust exposure [1,7–9], including a sugar beet refinery in which Forster *et al.* [1] showed specific IgG reacting with both sugar beet extract, and with airborne dust extract. In saw mills with high levels of mould exposure, Eduard *et al.* [10] found high titres of IgG reacting with mould antigens in the employed wood trimmers. In the latter study, the specific immune response was also related to the occurrence of respiratory symptoms [11]. To our knowledge, no attention has thus far been given to the role of certain IgG subclasses in these occupational studies.

This report focuses on specific IgG and IgG₄ responses and the relationship with respiratory health in 131 workers of four potato processing plants during the so-called 'campaign' from August to March [6] in the Netherlands. The results were obtained as part of a study in which many other exposure and effect parameters were investigated as well. Serological responses were related to personal antigen exposure levels, and a preliminary biochemical characterization of the antigens was performed.

Material and methods

Subjects of this study were 131 male employees from four facilities of a large potato processing company. Blood samples were drawn just before or at the start of the processing campaign (August 1992), and during the campaign (i.e. 7–17 weeks later in the period October–December 1992). Blood samples were taken from 109 workers in both periods. All subjects completed a questionnaire on occupational history, smoking habits and (work-related) respiratory and systemic symptoms. Control sera were obtained from 10 bakery workers [12], six members of a University Department and 20 randomly chosen healthy blood bank donors (with no information on age, gender and occupation).

Measurement of specific antibodies

Specific IgG and IgG₄ were determined by Enzyme Immuno Assay (EIA), with a coating of pooled airborne dust extract from one of the four plants [13] with a protein content of 150 µg/mg lyophilized material, as determined with the Bicinchoninic Acid reagent (Pierce, Rockford, USA). Microwells were coated overnight at 4 °C with the antigen at a protein concentration of 4.5 µg/mL. Sera were tested at 1/500 and 1/5000, both in duplicate, in phosphate buffered saline (PBS) containing 0.05% v/v Tween-20 and 0.2% w/v gelatin (PBTG; pH 7.4). After incubation for 1 h at 37 °C, binding of specific IgG and IgG₄ was determined by incubation for 1 h at 37 °C with peroxidase-labelled monoclonal mouse anti-human IgG or anti-human IgG₄ (Central

Laboratory of the Blood Transfusion Service, Amsterdam, the Netherlands), respectively, diluted 1:1000 in PBTG, and finally an incubation for 30 min at 20 °C with 2 mg/mL o-phenylenediamine (Sigma, St Louis, USA) in citrate/phosphate buffer, pH 5.5, containing 0.015% v/v hydrogen peroxide. Optical density (OD) was read at 405 nm. Control sera were tested in the same way at 100-fold and 500-fold dilutions.

Antibody concentrations were expressed as relative titres, calculated by interpolation of the logit-transformed OD-values on a calibration line obtained in the same microtitre plate with a pool of 18 strongly positive sera [13], which was arbitrarily given an antibody titre of 100. The absolute titre of the pool, defined as the dilution leading to an OD of 0.25, was about 1/5000. As the detection limit we used the titre interpolated from a logit value –3. For samples with a logit value above +3, the analysis was repeated at 5000-fold and 50 000-fold dilutions.

Characterization of antigens

An IgG₄ inhibition assay [13] was performed with extracts prepared from peeled raw potatoes as used in the potato processing company, and with potato allergen preparations provided by Laboratorium Diephuis' (ALK Benelux, Groningen, the Netherlands). The latter were obtained from washed unpeeled raw consumption potatoes.

Characterization of antigenic determinants was performed by pre-incubation of airborne dust extract at different temperatures (20, 37, 60, 80 and 100 °C) for 1 h, and by pre-incubation with trypsin, pronase or periodate as described by Doekes *et al.* [14].

Personal antigen exposure

Repeated personal full-shift measurements of inhalable dust were performed [6]. Filters were eluted as described by Houba *et al.* [15], and antigenic activity was measured in an IgG₄ inhibition assay [13]. Samples were tested at fivefold dilution in duplicate. Antigenic activity was expressed in Relative Antigen Units (RAU), with the pooled airborne dust extract used as the standard, and arbitrarily given a value of 100 RAU/mL.

Results

Levels of specific antibodies

In Figs 1a and 1b the titres are presented of specific IgG and IgG₄ against airborne dust antigens, in workers from the four plants (during campaign), in bakers and in other controls. Specific IgG was detectable in 95% and 100%, and IgG₄ in 50% and 0% of the workers' and

control sera, respectively. Antibody levels in potato workers' sera were higher than in control sera (Mann-Whitney *U*-test; $P < 0.01$ for IgG and $P < 0.001$ for IgG₄). Four new workers with no history of potato processing work showed low IgG levels not different from the controls, and no detectable IgG₄ levels. No association between appointment duration in the potato industry and antibody level was found. There was a strong association between specific IgG and IgG₄ levels for all 117 sera collected during the campaign (Spearman's correlation coefficient R being 0.80; $P < 0.0001$). This association was weaker, but still highly significant, when the analysis was restricted to the 58

sera with detectable IgG₄ and IgG levels ($R = 0.62$; $P < 0.0001$). Figures 1a and 1b further show remarkably large differences in specific antibody levels between plants, being more pronounced for IgG₄.

In the group of 109 workers from whom serum samples had been obtained both at the start and during the campaign, a small but significant increase during the campaign (on average 12%) was observed of both specific IgG (paired *t*-test; $P < 0.01$) and specific IgG₄ ($P < 0.005$) titre against airborne dust antigens. There was no relationship between time since the start of the campaign and change in IgG or IgG₄.

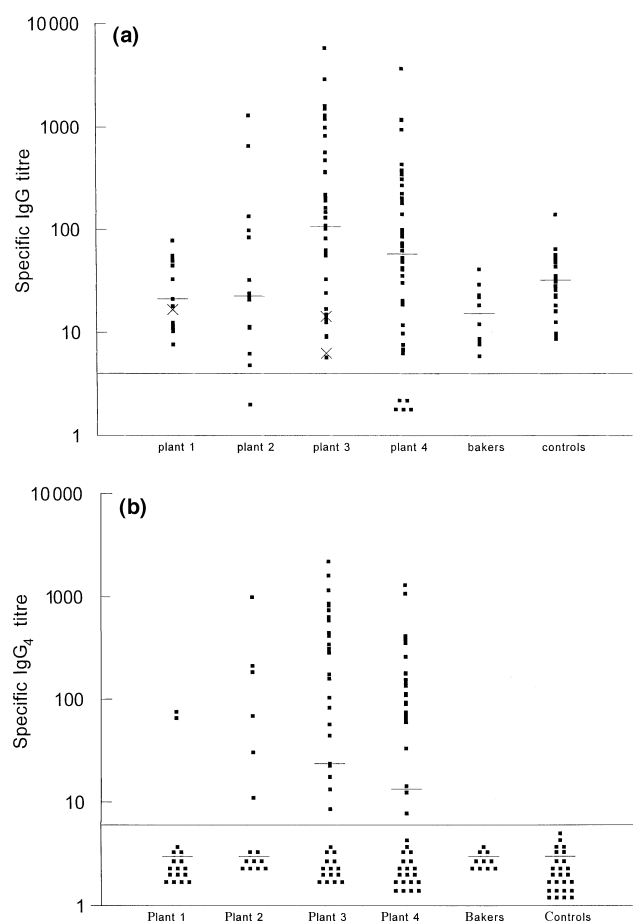


Fig. 1 (a). Titres of specific IgG against airborne dust antigens in 117 sera from potato processing workers (during the potato processing campaign), 10 bakers and 26 other controls. Median levels are marked with a '—'. Newly hired workers are marked with a 'X'. A titre of 4, indicated in the figure, was used as the detection limit. (b). Titres of specific IgG₄ against airborne dust antigens in 117 sera from potato processing workers (during the potato processing campaign), 10 bakers and 26 other controls. Median levels are marked with a '—'. A titre of 6, indicated in the figure, was used as the detection limit.

Characterization of antigens

Inhibition EIA's were performed to assess nature and biochemical characteristics of the antigens. A commercially available potato extract and an extract prepared in our own laboratory both inhibited IgG and IgG₄ reactions with the airborne dust extract completely, with dose-response inhibition curves that were parallel to those of the dust extract. This indicates that the large majority of the airborne dust antigens were potato components. The inhibitory capacities — defined as the protein concentration required for 50% inhibition — were similar for IgG₄, as shown in Fig. 2. For IgG, inhibitory capacities of the potato preparations were about 50% lower than of the dust extract (results not shown).

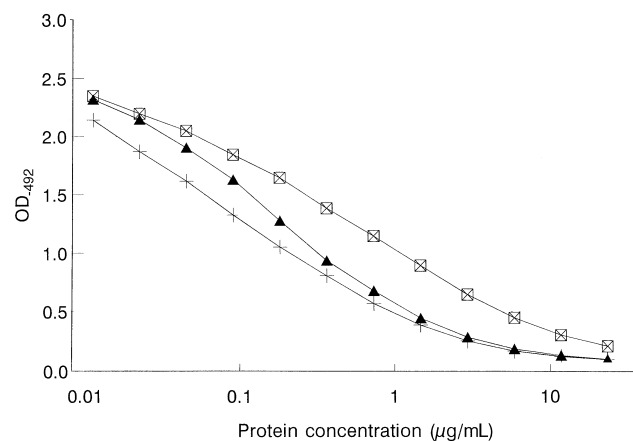


Fig. 2. Specificity of IgG₄ antibodies reacting with airborne dust extract from the potato processing industry. Inhibition EIA with a coating of airborne dust extract from the company studied, and IgG₄ antibodies in a pool of workers' sera. Inhibition curves are shown for a commercial potato extract (X), a potato extract prepared in the laboratory (+) and airborne dust extract itself (▲). OD-values of 0% and 100% inhibition are shown with a 'X'.

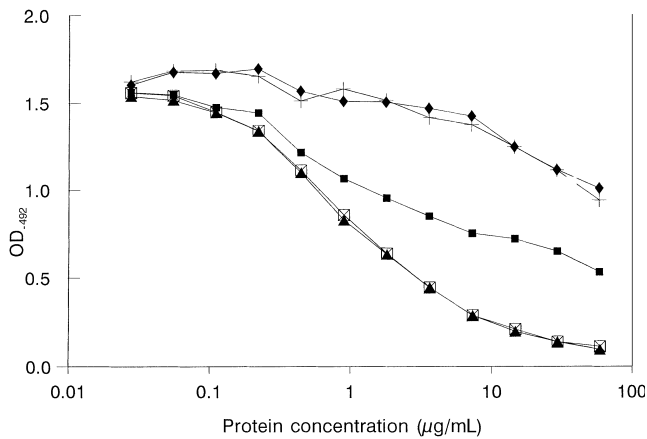


Fig. 3. Temperature sensitivity of IgG₄-binding antigens in airborne dust extract. IgG₄ inhibition EIA with a coating of airborne dust extract, and the same dust extract as inhibitor, after pre-incubation at 20° (□), 37° (▲), 60° (■) 80° (+) and 100 °C (◆). OD-values of 0% and 100% inhibition are shown with a 'X'.

IgG₄ inhibition EIA's were also performed with airborne dust extract after pre-incubation at different temperatures to study temperature sensitivity of the antigens; results are shown in Fig. 3. Antigenic activity was lower after pre-treatment at 60 °C compared with 20° and 37 °C. At 80° and 100 °C most antigenic activity was lost. Pre-incubation of airborne dust extract with trypsin, pronase or periodate, had no significant effect on the antigenic activity in a subsequent IgG₄ inhibition EIA (results not shown).

Personal antigen exposure and immune response

Antigen measurements were performed in 186 personal dust samples from 123 workers, and showed a wide range in

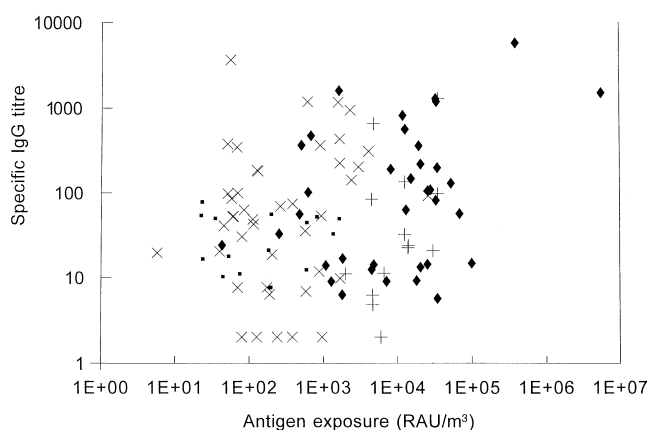


Fig. 4. Relationship between antigen exposure and specific immune response: scatterplot of specific IgG titre against airborne dust antigens, vs personal antigen exposure ($n = 109$, Spearman's $R = 0.23$). ■. Plant 1. +. Plant 2. ◆. Plant 3. ×. Plant 4.

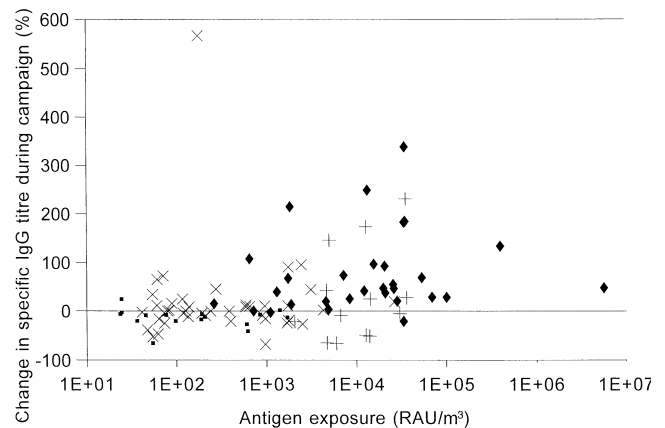


Fig. 5. Relationship between antigen exposure and specific immune response: scatterplot of increase of specific IgG titres against airborne dust antigens during the potato processing campaign, vs personal antigen exposure ($n = 101$, Spearman's $R = 0.43$). Titres below the detection limit were set at 2 before calculating the percentage change. ■. Plant 1. +. Plant 2. ◆. Plant 3. ×. Plant 4.

exposure levels. Large differences in exposure between the four plants were found, with the highest exposure levels in plants 2 and 3. In Fig. 4, a weak but significant ($P < 0.05$) association between specific IgG level during the campaign and mean personal antigen exposure level can be seen (Spearman's $R = 0.23$). For IgG₄, similar results were found ($R = 0.29$; $P < 0.005$). A stronger association ($R = 0.43$; $P < 0.0001$) with exposure level was found for the relative change in IgG titre during the campaign. In Fig. 5 the relative change in IgG during the campaign is shown vs antigen exposure. For IgG₄, similar results were found ($R = 0.45$; $P < 0.0001$). When analyses were restricted to workers without detectable specific IgG₄ to airborne dust antigens, no significant relations between antigen exposure and specific IgG level were found. From Fig. 5, a threshold level for an increase of at least twofold in IgG of about 1000 RAU/m³ might be suggested.

Workers were grouped according to their mean antigen exposure into three categories using 50- and 75%iles, corresponding with about 1000 and 10 000 RAU/m³, arbitrarily as cut-off levels. In Table 1 percentages of subjects with an increase of specific IgG or IgG₄ titre are shown for the three exposure categories. Results are shown separately for smokers and non-smokers, because current smokers showed 3–4 times lower titres of specific IgG and IgG₄ compared with never- and former smokers. A dose-response relationship for both groups can be seen, being stronger for non-smokers, as indicated by the χ^2 values. Relative increase in IgG₄ titre was higher for non-smokers ($\chi^2 = 11.0$; $P < 0.01$). For IgG, the relative increase was also higher ($\chi^2 = 2.6$), but not statistically significant. The table

Table 1. Percentages of workers with an increase of the specific IgG titre and IgG₄ titre during the processing campaign in three categories of antigen exposure using 50- and 75%iles as cut-off levels. Results are shown separately for smokers (*n* = 43) and non-smokers (*n* = 58)

Antigen exposure	<i>n</i>	Smokers		<i>n</i>	Non-smokers	
		Δ(IgG)>0	Δ(IgG ₄)>0		Δ(IgG)>0	Δ(IgG ₄)>0
Low	24	25%	13%	25	46%	31%
Medium	10	50%	0%	15	73%	73%
High	9	67%	56%	17	94%	82%
χ ² for trend		5.2*	5.3*		9.1**	11.9**

* *P* < 0.05. ** *P* < 0.01.

further shows more frequent increases in specific IgG, compared with specific IgG₄.

In multivariate analyses, factors were highly correlated and it was not possible to identify which were the most important influences on IgG and IgG₄. Smoking appeared to be an effect modifier which meant that a stratified analysis had to be performed, but in each stratum the number of study objects was then too small to permit the examination of many factors at a time.

Immune response and work-related respiratory symptoms

Sixteen per cent of the workers experienced at least one of the following work-related symptoms: cough (7%), phlegm (4%), shortness of breath (12%), wheezing (4%) and chest tightness (10%). These symptoms were reported by 18% of the current smokers and by 13% of the non-smokers. Titres of specific IgG in sera from workers with respiratory symptoms were lower than in sera from symptom-free workers, and 41% of the symptomatic workers had detectable specific IgG₄, compared with 54% of the symptom-free workers. Similar results were found for the specific work-related symptoms, but none of the differences was statistically significant (*P* > 0.05). No association was found between antigen exposure and respiratory symptoms, either. Thus, no positive association could be demonstrated between antigen exposure or specific IgG immune response and respiratory symptoms.

Discussion

In this study, a strong humoral immune response to airborne dust antigens was demonstrated among potato processing workers. Specific IgG was found in nearly all workers, and the IgG₄ subclass was found in about half of the workers. Control subjects with no history in potato processing work, and newly hired workers had low specific IgG levels and no

detectable IgG₄. Characterization experiments revealed that the antigens involved were potato components, most probably heat-labile proteins. This indicates that work-related inhalatory exposure to potato dust may result in a specific IgG response to potato antigens, in which specific IgG₄ antibodies seem to predominate. The absence of periodate sensitivity indicates that probably no carbohydrate structures were responsible for antigenic activity. However, the antigen activity was also not affected by the proteolytic enzymes trypsin and pronase. For trypsin, this can be explained by the fact that potato tubers contain considerable amounts of trypsin-inhibitor [16], which strongly reduces the trypsin activity. For pronase, no direct explanation can be given, but it is suggested that potato tubers also contain other protease inhibiting proteins [17,18].

Specific humoral immune responses to occupational antigens have been reported for other industries [1,8–10,19] as well. In this particular case it is noteworthy that antibody levels in controls are negligible while in the Netherlands potato consumption is very common. The heat-lability of potato antigens, shown in Fig. 3, may explain this apparent discrepancy since potatoes are usually cooked before consumption. The heat-lability of the antigenic proteins has also been described for the allergens causing IgE mediated potato allergy [20,21]. Specific IgG₁ and IgG₄ antibodies against potato were nevertheless detectable in a significant number of sera from 213 unselected Dutch children, aged 3 months–14 years [22], although specific IgG₄ levels to potato were in general low compared with other foods and compared with specific IgG₁. The used IgG₄ radioallergosorbent test (RAST), however, had a much lower detection limit compared with our study. For wheat flour antigens, specific IgG₄ antibodies can be found in individuals with an occupational airborne antigen exposure, namely bakery workers, but also in 15–30% of controls [19]. Thus wheat components might induce specific IgG₄ antibodies both after bread consumption and after wheat flour

inhalation, whereas IgG₄ anti-potato antibodies only occur after inhalatory exposure.

A weak, but significant, relationship between antigen exposure and specific immune response was noted, and appeared to be mainly determined by specific IgG₄ responses. This corroborates our suggestion that the IgG₄ subclass accounts for the greater part of the specific IgG response. The relative change in specific antibody levels in the course of the campaign was stronger related to antigen exposure than the titre itself, probably because the specific antibody levels reflect the effect of several years of exposure. This assumption is supported by the absence of specific IgG₄ in newly hired employees after only a few months of exposure. Change in IgG by antigen exposure showed a dose-response relationship between exposure and change in specific IgG. The correlation was stronger for non-smokers, suggesting that smoking is an effect modifier in this association. A multivariate analysis including smoking status as independent variable was therefore not appropriate. It is known from the literature that current smokers have lower serum IgG antibody levels [23,24], but it is notable that smoking also modifies the effect of antigen exposure on specific humoral immune response.

Although antigen exposure was similar in plants 1 and 4, specific IgG₄ titres were much higher in plant 4. The number of current smokers, 48% and 40%, respectively, was not essentially different and cannot explain the observed differences. Endotoxin exposure levels, however, appeared to be on average three times higher in plant 4 compared with plant 1 [6], and this might be of importance for the observed differences in IgG(4) titres.

A positive relationship between specific IgG and work-related symptoms was not found, and type III allergy is thus unlikely to play a predominant aetiological role. Moreover, IgG₄ appeared to predominate the specific immune response, and IgG₄ is neither able to react as a precipitating antibody in type III allergy nor to activate the complement system [25]. The levels of IgG(4) in symptomatic workers tended to be somewhat lower in non-symptomatic workers; a protective effect of IgG₄ as 'blocking antibody' [25] can therefore not be excluded. This protective effect has also been observed among pig farmers [26]. Positive associations between specific IgG antibodies and respiratory symptoms, suggesting extrinsic alveolitis, have been observed in other settings [11,27], but the role of the IgG₄ subclass has not yet been elucidated.

We conclude that occupational respiratory exposure in the potato processing industry leads to a strong humoral immune response, which is mainly directed to potato antigens, probably heat-labile potato proteins, and is predominated by a specific IgG₄ response. This response, however, is probably of no relevance for the occurrence of respiratory

symptoms. The presented data even suggest that specific IgG₄ production might protect against health effects.

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