Risk modification and combined exposures in occupational respiratory allergy

Risico modificatie en gecombineerde blootstellingen bij beroepsgebonden allergische luchtwegaandoeningen (met een samenvatting in het Nederlands).

Proefschrift ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen, ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen op Dinsdag 28 September 2004 des middags om 12.45 uur

door

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geboren op 11 mei 1964, te Voorschoten

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The studies described in this thesis were financially supported by the " Nederlands Astma Fonds".

Printed by: PrintPartners Ipskamp B.V., Enschede

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PhD thesis Utrecht University. With references and a summary in Dutch.

ISBN 90-393-3802-7

Abstract

The main objective of the studies described in this thesis was to investigate the impact of combined exposure to allergens and non-allergenic agents on the development of respiratory allergy in occupational populations. The effect of early life exposure to the farming environment and endotoxin exposure on IgE mediated sensitization to common allergens (atopy) and respiratory symptoms was studied in farming students and adult pig farmers. Both a farm childhood and current exposure to the farming environment were associated with a much lower prevalence of atopy in farming students and non-farming controls. A low risk of sensitization to common allergens was also found in full time pig farmers, and was associated with chronic high dose exposure to endotoxin. Whether the reduced prevalence of atopy also affects risk of (allergic) respiratory disease is not clear from these studies. A farm childhood was associated with a lower prevalence of wheeze, doctor-diagnosed asthma, and bronchial hyper-reactivity (BHR), but current farming and high endotoxin exposure were associated with higher prevalences of respiratory symptoms and increased BHR in farming students and pig farmers.

The relation between disinfectant exposure and respiratory allergy was studied in pig farmers and in laboratory animal (LA) workers that had been working with LA's for more ('experienced') or less ('novice') than 4 years. Results from the study in pig farmers confirmed that disinfectant use might be a risk factor for allergic respiratory disease in this population. Prevalence of sensitization to common allergens was very low in farmers who did not use disinfectants, but strongly increased with increasing frequency of disinfectant use. Disinfectant use was also a risk factor for respiratory symptoms in atopic pig farmers, which concords with earlier findings, and was associated with signs of upper airways inflammation. Nevertheless, prevalences of sensitization to common and laboratory animal allergens were lower in 'novice' LA workers using disinfectants containing chloramine-T, and use of disinfectants other than alcohol was associated with lower prevalences of respiratory symptoms to common allergens and laboratory animals. However, no association was found between disinfectant use and sensitization or respiratory symptoms in 'experienced' workers, and there was some indication that the inverse association in 'novice' workers might be due to reversed causation.

We also investigated whether high titers of IgG4 antibody to rat urinary allergens (RUA) could be a marker for the induction of immunological tolerance in LA workers. Exposure to RUA was associated with high titers of allergen-specific IgG4 antibody. However, IgG4 was a strong risk factor for both prevalent and newly occurring sensitization and symptomatic allergy to rats in atopic and rat-sensitized workers respectively. High IgG4 to RUA is therefore not a marker for the induction of tolerance, but rather reflects aspects of exposure and susceptibility.

Long-term changes in lung function in workers exposed to high molecular weight sensitizers were assessed using follow-up data from the study in LA workers. Contact with laboratory animals was a

significant risk factor for accelerated annual lung function decline, but this was most pronounced in sensitized workers who continued to be exposed to the animals they were sensitized to.

Results from these studies illustrate the impact of combined exposures and interaction effects on the development of occupational respiratory allergy. Although allergen exposure is probably the most important determinant of IgE sensitization or symptomatic allergy in susceptible workers, exposure to non-allergenic agents such as endotoxin or disinfectants may significantly affect susceptibility. As combined exposures are the rule rather than the exception in the working environment, these effects are relevant to risk assessment of both allergens and non-allergenic agents.

Contents

Chapter 1

Introduction

Occupational respiratory allergy may be described as a *newly* occurring disease of the lower or upper airways that results from allergen-specific immune responses to agents encountered exclusively or predominantly at the workplace. This definition includes newly occurring asthma and rhinitis, but explicitly excludes work-aggravated disease where respiratory symptoms are triggered by work-related exposures (inciters) in subjects with pre-existing allergies. Many occupational sensitizers with a high molecular weight (HMW) induce strong allergen-specific IgE antibody responses, and it is generally thought that these antibodies are etiologically involved in the manifestation of clinical allergy to these agents. For many low molecular weight (LMW) agents (e.g., isocyanates, plicatic acid) allergen-specific IgE antibodies are not detectable or found in only a small proportion of symptomatic workers.[1] The specificity of responses observed in provocation studies and the latency period between exposure and symptom development suggest, however, that immune-mediated sensitization is etiologically involved for these agents as well.[2, 3] For the remainder of this thesis the emphasis will be on IgE-mediated respiratory allergies to agents with a high molecular weight. Commonly recognized subcategories include indoor allergens (e.g. house dust mite, pet allergens), outdoor allergens (e.g. tree and grass pollen), and work-related allergens (e.g. enzymes, latex, cereal flours, laboratory animal allergens).

Towards the end of the 20th century occupational asthma has replaced pneumoconiosis as the leading cause of chronic respiratory morbidity in the working population. This is at least partly due to the improved prevention of pneumoconiosis, but there are indications that prevalence and incidence of occupational asthma have increased.[4] Based on figures from voluntary reporting schemes and medico-legal statistics the incidence of occupational asthma has recently been estimated to be between 2-15 cases/100.000 persons per year.[5] Rates from medico-legal statistics probably underestimate the actual incidence of occupational asthma, as diagnostic criteria are usually rather strict and may include non-medical criteria, while only suspected sensitizers are covered. In epidemiological studies the proportion of adult-onset asthma cases that can be attributed to workplace exposures has been variously calculated to be between 10-25%.[6] These attributable risk estimates are almost certainly an overestimate, as cases of work-aggravated asthma will have been included.

Dose-response modeling

Although rarely fatal nowadays, allergic airway diseases are of great health concern because of the high morbidity and usual poor reversibility. Factors associated with a positive outcome of occupational asthma are early detection and complete removal from exposure, although this may not lead to complete cessation of respiratory symptoms.[7]

Sensitization to work-related allergens and work-related rhino-conjunctivitis are strong predictors for the development of asthma symptoms, although this has rarely been assessed in longitudinal studies.[8, 9] However, studies in the general population have also shown a strong relation between presence of rhinitis symptoms and asthma incidence during follow-up[10, 11], and subjects with work-related rhinitis who continue to be exposed may therefore be at increased risk for developing asthma. Prevention of work-related sensitization or symptomatic allergy must therefore be the primary goal from a public health point of view. This requires estimation of dose-response relations for allergen exposure and selected health effects and identification of factors that may significantly affect these relations.

Health effects

It has been argued that the preferred endpoint in dose-response studies should be a clinical definition of occupational asthma.[12] This would neglect other common adverse outcomes of allergen exposure such as rhinitis, and require large studies as the incidence of occupational asthma is in the order of 2-15 cases/100.000 persons per year. Clinical assessment of workers in an occupational setting is expensive and time-consuming, although this may be reduced by stepwise ascertainment of cases. A choice for allergen-specific sensitization as an endpoint has been criticized on the grounds that it is not an adverse health effect and is sometimes only weakly associated with presence of respiratory symptoms.[12] However, IgE mediated sensitization can often be shown highly specifically, and is much less subject to differential misclassification than a self-reported history of respiratory symptoms. An alternative would be the presence of symptoms consistent with asthma and/or rhinitis in combination with evidence for allergen-specific sensitization.[13-15] The dose-response curve that is obtained is the result of superimposing separate dose-response curves for the relation between allergen exposure and sensitization, and for the relation between allergen exposure and symptomatic allergy in sensitized workers.[16] Dose-response relations found in the studies referred to above tended to be dominated by the relation between allergen exposure and symptomatic allergy in sensitized workers.[14, 15] Although this may not be a problem for risk assessment purposes per

se, it does not allow identification of risk factors or effect modifiers for sensitization or symptomatic allergy separately. As longitudinal studies have shown that sensitized workers who do not report symptoms are at increased risk for becoming symptomatic during follow-up[8, 9, 15], it might be better to study the relations between exposure and sensitization or respiratory symptoms separately, allowing for the possible modifying effects of sensitization on symptom development in the analysis.[15]

Allergen exposure

Given the causal relation between allergen exposure and allergy, it is not surprising that epidemiological studies in occupational groups over the last decades have demonstrated that the level of allergen exposure is an important determinant for development of specific IgE mediated sensitization and symptomatic allergy.[15, 17-25] Lack of adequate measurement techniques have long hampered more precise quantitative estimation of dose-response relations between allergen exposure and respiratory allergy, and exposure threshold limit values have therefore been formally proposed for only two allergens, subtilisin and wheat flour.[26] Improvements in sampling equipment and sampling techniques, more refined statistical modeling of exposure, and development of sensitive and allergen-specific immunoassays have all enabled investigators to describe quantitative dose-response relations for exposure to several allergens and specific IgE mediated sensitization or symptomatic allergy.[12, 16] These include many of the HMW allergens responsible for a large proportion of work-related asthma cases, i.e. rat urinary allergens[15, 22], wheat flour[20], and alpha-amylase.[19, 23] One notable exception is latex for which no clear dose-response relation between exposure and allergy has yet been found.[27] Specific immunoassays for latex allergens have only recently been developed, and airborne latex allergen exposure has therefore been assessed in only a few studies.[28-30]

It has become clear that the sensitizing potential of different allergens may vary widely. An increased prevalence of sensitization to rat urinary allergens has been observed at an estimated 8-hour time-weighed average (TWA) exposure below 1 ng/m^3, whereas no significant increase in prevalence of sensitization to wheat allergens could be observed at exposures up to 100 ng/m^3 (8-hour TWA).[31] It is not known what exposure characteristics (e.g., average exposure, peak exposure, exposure duration) are most important for developing respiratory allergy. However, theoretical considerations of the particle sizes involved as well as actual measurements indicate that there is a close correlation between (summed) peak

exposure and average exposure.[16, 32] Several studies have shown clear exposure-response relations for allergen exposure and sensitization or symptomatic allergy when exposure was defined as average exposure or as the product of average exposure and weekly duration of exposure.[13, 15, 19-23, 25]

Information on background risks of sensitization or respiratory allergy for occupationally non-exposed individuals is lacking for most allergens. An excess risk of occupational low-level exposure can therefore not be accurately determined and existence of a no-effect-level not be (dis)proven.[14] For most allergens the exact shape of the dose-response curve is unknown, but there are indications that the risk of sensitization or symptomatic allergy may level off or even decrease at high (and/or continued) exposure for some allergens.[14, 21] This could be due to the induction of immunological tolerance by high-dose allergen exposure[33], but could also result from exposure avoidance by susceptible workers or from selective removal of highly exposed symptomatic workers from the working population.[14, 34]

Susceptibility and exposure interaction

A diagram outlining the main pathogenic events in the development of occupational respiratory allergy is presented in figure 1. Susceptibility may be due to the presence of specific susceptibility genes, exposure to environmental risk factors, or both. Allergen exposure may result in the production of allergen-specific IgE antibodies (sensitization) in susceptible workers, and this can be influenced by concurrent exposure to non-allergenic agents from the general and occupational environment. Continued exposure to the allergen may lead to symptomatic allergy in sensitized workers, but symptoms can also be triggered by exposure to non-allergenic agents. Although not explicitly indicated in the diagram, genes and exposures that determine susceptibility or allergen-specific sensitization may be different from those that affect development of symptomatic allergy. For most allergens only a proportion of exposed workers become sensitized or symptomatic, even at high exposure levels, which suggests that there are large differences in susceptibility between workers.[35]

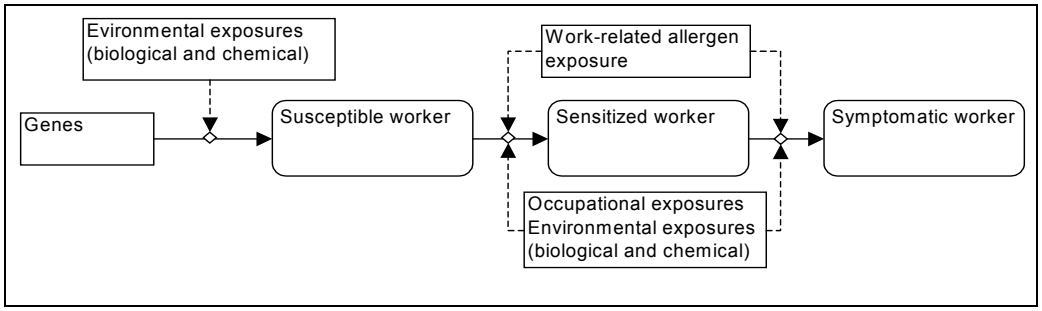

Figure 1. Gene-environment interactions and combined exposures in the pathogenesis of occupational respiratory allergy.

Timing of exposures also has to be considered, as the interaction between genes, environmental factors, and allergen exposure may be different during early life, may depend on exposure history, and may be different during early phases of the immune response to an allergen.[36]

It is important to take proper account of interactions when studying dose-response relations between allergen exposure and sensitization or symptomatic allergy, as they can provide clues on potential etiological mechanisms and help identify susceptible groups in which preventive actions may be more effective.[37]

An overview of risk factors for occupational asthma is available from several review articles,[3, 35, 38-43] and therefore only factors that have been shown or are likely to be strong risk modifiers for the relation between allergen exposure and occupational respiratory allergy will be discussed more extensively.

Genetic factors

Studies in the general population have shown that allergy and asthma have strong hereditary components, and indicate that multiple genes are involved.[44] There is evidence for strong gene-environment interactions[45], which may be more pronounced during early life when both the immune system and the respiratory tract are still developing.[46, 47]

Genetic studies in occupational populations have the advantage that the phenotype can be defined more clearly, and that it is easier to assess potential gene-exposure interactions for allergen exposure.[48] The majority of genetic studies in occupational populations have investigated the association between human leukocyte antigen (HLA) class II polymorphisms and risk of sensitization or asthma to LMW allergens.[49] For some of these agents, the contribution of HLA class II alleles appears to be greater in those that are less exposed.[50]

HLA class II polymorphisms have recently also been associated with prevalence of sensitization to rat urinary allergens and work-related chest-symptoms in laboratory animal workers[48, 51] and with prevalence of specific IgE antibodies to the latex allergen hevein in health care workers with latex allergy.[52]

Host factors

Atopy has been defined as the propensity to produce IgE antibody to allergens that are commonly encountered in the general environment.[53] In epidemiological studies atopy is often defined as the presence of a positive skin prick test (SPT) or IgE antibodies to at least one of a series of common environmental allergens. The atopic phenotype has long been considered to be a relatively stable, even life-long, attribute[54], but recent studies have shown that the expression of atopy may be significantly modulated by environmental exposures.[55] Atopic sensitization to common allergens is a recognized risk factor for work-related sensitization and occupational asthma due to HMW allergens, although the positive predictive value for occupational asthma is not very high. Screening for atopy as a preventive measure is therefore discouraged.[4, 34] Atopy has been shown to be a risk modifier for the relation between allergen exposure and allergen-specific sensitization (either alone or in combination with respiratory symptoms) in a number of cross-sectional studies. Dose-response curves were steeper for atopic workers in occupational populations exposed to laboratory animal allergens[22] and wheat flour.[14, 20] In a longitudinal study reported by Gautrin et al. (2002), a dose-response relation between hours of exposure to rodents and allergen-specific sensitization or work-related symptoms was found only in non-atopics, while risk in atopics was high, but independent of exposure.[56]

Smoking is associated with sensitization and development of occupational asthma in workers exposed to platinum salts and anhydrides.[57-63], but does not seem to affect the risk of sensitization to HWM allergens.[15, 24, 25, 31] Age and sex also appear not to be related to development of occupational allergy to HMW allergens.[20, 21, 25]

Environmental and occupational exposure to non-allergenic agents

Both animal and human experimental studies have shown that exposure to non-allergenic air pollutants, such as ozone, diesel exhaust particles, and NO, may enhance existing immune responses to common inhalant allergens.[64-73] Recent studies have also shown that exposure to

environmental tobacco smoke and diesel may have an adjuvant effect, and stimulate the initiation of IgE responses to allergens.[74-76]

Combined exposure to allergens and other non-allergic agents is a feature of many work environments. Many occupations involve exposure to organic dusts containing microorganisms and endotoxin that may have strong pro-inflammatory properties by activating non-specific innate immune responses. Exposure to dust containing endotoxin has long been recognized as an important cause of asthma-like symptoms in cotton workers, livestock farmers, and grain handlers.[77] Endotoxin has strong pro-inflammatory properties, and several studies have shown that endotoxin exposure can aggravate asthma symptoms in both adults and children.[77] In pig farmers, a strong relation between endotoxin exposure and respiratory symptoms, decreased lung function, and increased lung function decline has been observed.[78, 79] Recently, the apparent protective effect of growing up on a farm on atopic sensitization and symptoms of respiratory allergy in children and young adolescents has been ascribed to endotoxin exposure.[80, 81] It is uncertain whether this possible protective effect of childhood endotoxin exposure on atopy and respiratory allergy extends into adulthood, or whether high endotoxin exposure may also protect from allergic sensitization and respiratory allergy in occupationally exposed populations. The relation between occupational exposure to specific chemical agents and allergic sensitization has been evaluated in only few studies. Exposure to disinfectants containing quaternary ammonium compounds (QAC's) was associated with a higher prevalence of atopic sensitization to at least one of four common allergens in a case-control study among pig farmers.[82] Intriguingly, a chemically related QAC (dimethyldioctyldecyl ammonium bromide) has been used as an adjuvant in vaccination studies, although because of its stimulating effect on Th1 immune responses.[83, 84]

Impact of risk modification on study design and statistical analysis

As discussed above, the interaction between genetic factors, allergen exposure, exposure to non-allergenic agents, and the timing of exposures may all be relevant to the development of respiratory allergies in occupational populations. This can make interpretation of results obtained in cross-sectional studies extremely difficult, and a longitudinal design is therefore strongly preferred. Reanalyzing data from already completed studies often results in inconclusive findings, as they usually lack power because they were not designed to detect interaction effects. However, when strong effect modification is present, stratified analyses of already collected data may uncover associations that were not apparent before.

Aim and structure of the thesis

The main objective of this thesis is to study the impact of combined exposure to allergens and non-allergenic agents on the development of respiratory allergy in occupational populations. For most of the analyses data were used that were available from existing studies that investigated risk factors for laboratory animal allergy and development of respiratory symptoms in farming students and pig farmers. One study was designed with the purpose of investigating the impact of disinfectant exposure on respiratory allergy in pig farmers.

Although pig farmers are exposed to high levels of animal allergens, the prevalence of work-related sensitization is generally low. This has been attributed to low allergenic potency of pig urinary proteins, but could also be explained by protective effects of other farm-related exposures. In Chapter 2 we investigate whether a farm childhood and current farming are associated with IgE mediated sensitization and respiratory symptoms in young adults starting a career in farming. Data are from a follow-up study among Danish farming students and conscripts from the same rural areas as controls. Prevalence of sensitization is compared for those who were brought up on a farm and those who were not and for farming students and controls.

In Chapter 3 we further investigate whether the apparent protective effect of the farm-environment can also be found in adult full-time farmers and whether it is associated with endotoxin exposure. Data are from a case-control among Dutch pig farmers. Non-parametric regression analysis (smoothing) is used to study the shape of the dose-response relation between endotoxin exposure and atopic sensitization to common allergens in these farmers.

Disinfectant exposure has been identified as a potential risk factor for atopic sensitization and adverse respiratory health effects in pig farmers. In Chapter 4 we investigate the association between disinfectant exposure and prevalence of sensitization to common allergens, pig urine, and disinfectants, prevalence of respiratory symptoms, pulmonary function, and markers of airway inflammation. Data are from a cross-sectional study among Dutch pig farmers that was designed with the purpose to investigate the impact of disinfectant exposure on respiratory allergy.

Laboratory animal workers are known to be exposed to highly potent rodent allergens, while exposure to microbial agents is much lower than in pig farmers. A clear dose-response relation between exposure to rat urinary allergens (RUA) and allergen-specific sensitization and respiratory symptoms has been shown in this population. In Chapter 5 we examine the

association between disinfectant use and IgE mediated sensitization, respiratory symptoms, and lung function using data from a large longitudinal study in laboratory animal workers.

High-dose exposure to animal allergens may result in immunological tolerance, and this could possibly explain the slightly lower prevalence of sensitization to rat allergens and symptomatic rat allergy in highly exposed laboratory animal workers. In Chapter 6 we investigate whether presence of allergen-specific IgG4 antibodies to RUA is a marker for a protective immune response in laboratory animal workers. We analyze the relation between titers of IgG4 against RUA and both prevalent and incident sensitization and symptomatic allergy to rats.

Clinical studies in sensitized subjects suggest that continued exposure to sensitizing agents can lead to chronic loss in lung function in subjects with occupational asthma, but there is almost no information on long-term changes in lung function from follow-up studies in workers exposed to high molecular weight sensitizers. In Chapter 7 we study the relation between exposure to laboratory animals and longitudinal changes in lung function in sensitized or symptomatic laboratory animal workers.

Finally, in Chapter 8 we discuss the potential impact of combined exposure to allergens and non-allergenic agents on the development of allergy in occupational populations in relation to genetic factors, host factors, and allergen exposure, using data from the studies described in this thesis.

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Chapter 2

Low Prevalence of Atopy in Young Danish Farmers and Farming Students Born and Raised on a Farm

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SUMMARY

Background Recent studies have shown that in several countries atopic sensitization to common allergens (common atopy) and atopic symptoms are markedly less prevalent in children living on a farm, compared to non-farm children living in the same rural areas. Living conditions on farms may, however, vary largely between different countries. It is also not yet known whether the 'protective' effect of a farm environment can also be found in adults.

Materials and methods Common atopy and respiratory health were assessed by skin prick tests (SPT), questionnaire and measurement of bronchial hyperresponsiveness (BHR) in the SUS study, a cohort study on respiratory health in Danish farming students and conscripts from the same rural areas as controls. Results of SPT were confirmed by IgE serology in all SPT+ subjects and a subset of SPT- subjects. Prevalences of common atopy, respiratory symptoms and bronchial hyperresponsiveness were compared for farmers and controls, and for those who had or had not lived on a farm in early childhood.

Results In multiple logistic regression analyses adjusting for ever smoking and a familial history of allergy, both being a farmer (OR's 0.62-0.75) and having had a farm childhood (OR's 0.55-0.75) appeared to contribute independently to a lower risk of sensitization to common allergens as assessed by SPT and IgE serology. A farm childhood was also inversely associated with high total IgE (OR~0.68), presence of respiratory symptoms (OR's~0.69-0.79) and BHR (OR~0.61) in these analyses. Direction and strength of the association between being a farmer and respiratory symptoms or BHR varied widely (OR's~0.69-1.28).

Conclusion The "anti-atopy" protective effect of a farm childhood could be confirmed in Danish farming students: prevalences of positive SPT, specific and total IgE, allergic symptoms, and BHR were lower in those being born or raised on a farm. Past exposure to the farm environment in early childhood may therefore also contribute to a lower risk of atopic sensitization and disease at later age.

Clin Exp Allergy 2002; 32:247-53

Introduction

In several recent reports a markedly lower prevalence of atopy has been demonstrated in farm children, who appeared to have a 2-3 times lower risk of being atopic compared to their peers in the same rural areas and visiting the same schools but not living on a farm [1-5]. In these studies atopy was defined as self-reported symptomatic atopic disease including hay fever, doctor-diagnosed asthma or recurrent wheezing [1-5], and/or as type I sensitization to common, non-farm associated allergens like dust mites, pollens and pets as assessed by IgE serology [1] or skin prick tests [3,5]. In one study, non-specific airway hyperresponsiveness was also shown to be less prevalent among those who lived on a farm [5].

These findings are remarkable since the farm environment is well known for its high levels of exposure to airborne organic dusts containing a wide variety of antigenic and potentially allergenic proteins of plant, animal and microbial origin [6-9]. Since in traditional farming the work and home environment are usually closely associated, high allergen exposure levels are likely for all who live at a farm, also when not directly involved in farm work, such as children. Apart from typical farm-associated allergens, 'common allergens' like those from pets or house dust mites are usually also abundant in the farm home environment [10-12]. With regard to common outdoor allergens, like pollen and mould spores, it seems very likely that farmers and their family members are on average exposed to at least equal and probably higher allergen levels than non-farming subjects in a rural or urban environment.

Since farm environments may show considerable differences between various countries and/or regions, confirmation of these findings from different farming communities is of major importance. Although at least five such studies have been published, three came from the same geographic region – Switzerland [1], Bavaria [2], and Austria [3] -, and mainly involved children living at relatively small family farms with mixed types of agriculture. Three studies included skin prick tests (SPT) [3,5] or specific IgE measurements [1], while the Bavarian study was completely based on questionnaire data [2]. The Finnish study found the 'anti-atopy' effect of a farm childhood in a questionnaire study among first year university students [4], which suggests that the effect would last into young adulthood; unfortunately this study did not include objective tests for the presence of atopy (SPT or IgE serology).

We have investigated atopic sensitization to common allergens, symptoms of respiratory allergy, and bronchial hyperresponsiveness (BHR) in the SUS project, a Danish cohort study [13] in which respiratory health status and its development are studied in up to 2,000 farming

students and young farmers, and a control group of 400 conscripts coming from and living in the same rural areas. Data were analyzed with regard to where subjects had lived in childhood, and their current status as a farmer or farming student, or as a non-farming control.

Material and Methods

Population

In 1992-1994 all 2,478 second year students of farming schools ('farmers') in Denmark were invited to participate in the study, and at baseline 1,734 male and 230 female students were enrolled. A control group was recruited in 1994 of 407 conscripts of the Danish military service (controls), who all had indicated to live in rural areas, but were not currently involved in farm work, nor intended to start a farming career. Details of the recruitment and selection of study participants, response rates, as well as various relevant and general characteristics - like prevalence of smoking, percentage females, mean age, familial history of allergy, working experience with farm animals etc. - have been described in a previous report in which also the mean lung function and prevalences of various respiratory symptoms and the presence of bronchial hyperresponsiveness at baseline were analyzed [13]. Parents of farming students completed an additional questionnaire on smoking habits, family size and the prevalence of allergy among parents and grandparents.

After data on approximately 800 farmers had been collected, the baseline health and living environment questionnaire was modified to include a question on where the study subject had lived during childhood ("Have you been born and raised on a farm?"). Respondents were classified as "farm child" if they answered yes to being born and raised on a farm, and as 'no farm child' if they were not. In total 1028 male and 138 female farmers and all 407 controls answered this question.

Atopy : skin prick tests

All individuals were skin prick tested with 14 allergens, including the common - i.e. not specifically farm-associated – allergens house dust mite, cat, dog, pollen from grass (mix of five species), birch, and mugwort (*Artemisia*), and various common moulds. The panel further included known or presumed 'farm-related' allergens like animal allergens from pigs, cows and/or horses, and 3 different storage mites [13,14]. In all subjects reactions to a negative (saline) and positive (histamine 5 mg/ml) control were also assessed. A weal diameter of at

least 3 mm was regarded as a positive response, provided that the weal diameter of the negative control did not exceed 1 mm, and the weal diameter of the positive control was also at least 3 mm.

Atopy on the basis of positive skin prick tests was defined as at least one positive SPT to one of the five common allergens house dust mite, grass pollen, birch pollen, cat or dog; this parameter was designated 'SPT(5)'.

Atopy: IgE serology

Blood samples had been taken at the start of the study and serum had been frozen in aliquots at -80°C. Total IgE and specific IgE antibodies to the common allergens house dust mite, grass pollen (mix of 2 species), birch pollen, cat and dog, and to an allergen extract of storage mites (3 species) were measured by enzyme immunoassays [15]. Only a subset of sera was selected: from all subjects in the whole cohort who had shown at least one positive SPT to any of the 14 allergens in the test panel (n=768; 32%) and a random selection of 100 subjects with negative results for all tested allergens.

Allergen-specific IgE was assessed with 1/10 diluted sera in duplicate allergen-coated microwells. A serum was considered positive if the optical density at 492 nm in both wells exceeded the mean OD_{492} in the control wells by >0.050. If the OD_{492} in only one of the duplicate wells reached this level, the test was repeated. If the result of the second test was still inconclusive, the serum was considered positive if the mean value of both wells exceeded the mean OD_{492} in the control wells by >0.050. A total of 409 sera were retested on a second occasion, and a concordance of negative and positive findings of >90% was found, with practically all discordant results in sera with borderline reactions. Atopy on the basis of specific IgE tests - 'IgE(5)' - was defined as at least one positive test for one of the five common allergens.

Total IgE was measured by sandwich EIA, with sera diluted 1/10, 1/20 and 1/40, and as calibration standard the IgE standard for the Pharmacia CAP system [15]. Although total IgE levels in both populations showed a log-normal distribution, the values were dichotomized and 'high total IgE' was defined as a serum IgE level of >100 kU/L. A total of 406 sera were re-tested on a second occasion. The mean % difference between the two values found in this series was 13%; with regard to the classification as 'high' or 'normal', a concordance of 97% was found, with practically all discordant results for sera with total IgE levels between 80 and 120 kU/L.

Respiratory symptoms and bronchial hyperresponsiveness

The baseline health and living environment questionnaire included questions on respiratory symptoms and doctor diagnosed diseases. Wheeze was diagnosed when subjects reported having ever wheezed. Asthma was defined as described previously [13]. Non-work related rhinoconjunctivitis was diagnosed when subjects reported upper airway or eye symptoms, which were worse at home or after contact with pollen or in spring, summer, or autumn.

Bronchial hyperresponsiveness was measured as described previously [13] with calibrated DeVilbiss No 40 nebulizers (Somerset, PA, USA) delivering a cumulative dose of 1.44 mg histamine. Subjects whose FEV_1 fell by 20% or more of the largest FEV_1 recorded at baseline or after 0.9% saline were considered as having bronchial hyperresponsiveness (BHR).

Statistical analyses

All statistical analyses were performed using SAS (version 6.12). In order to obtain a satisfactory match with the rural controls (n=407) only male farmers aged under 26 were selected for further analysis (n=1691). Information on childhood residence, smoking behavior, and familial history of allergy were available for 999 male farmers (59%) and 402 controls (99%). BHR was measured in 987 male farmers and 380 controls. Due to the exclusion of subjects with a positive SPT reaction to the buffer control or a negative reaction to histamine, relations with SPT(5) could be analyzed in 988 male farmers and 399 controls. Since IgE was measured in a selected subpopulation the relations with IgE(5) and 'high total IgE' could be analyzed in only 302 male farmers and 143 controls. Alternative cut-off values were tested, but had only little impact on the associations reported in this paper. Combined effects of being a farming student ('farmer') and of a farm childhood, were analyzed in multiple logistic regression analyses (PROC LOGISTIC) with an adjustment for (ever) smoking and a familial history of allergy. No adjustment for age was made in this truncated sample of young persons. Results were calculated and expressed as Odds Ratios with 95% confidence intervals.

Results

Main population characteristics for both subgroups of farmers and for controls are given in Table 1. Controls were slightly older than farmers and more often reported a familial history of allergy. Within the group of farmers, farmers with a farm childhood smoked less often and tended to have more siblings than farmers without a farm childhood. Working experience

Table 1. Demographic characteristics of study population according to current working life as a farmer and living at a farm in childhood

	Controls (n=402)	Farmers, no farm childhood (n=494)	Farmers, farm childhood (n=505)
Smoker (%)	132 (32.8)	185 (37.5) †	116 (23.0)
Ex-smoker (%)	1 (0.3) ‡	22 (4.5)	12 (2.4)
Age (±sd)	19.5 (±0.9) ‡	19.2 (±1.2)	19.4 (±1.4)
Familial history of allergy (%)#	33 (8.2)	28 (5.7)	30 (5.9)
Normalized years with pigs§	0.0 (±0.0) ‡	1.0 (±2.3) †	2.0 (±5.1)
Normalized years with cattle§	0.0 (±0.0) ‡	1.7 (±2.8) †	4.8 (±5.3)
No. siblings (%)*†			
0		17 (4.2)	12 (2.7)
1		198 (48.6)	117 (26.7)
2		111 (27.3)	180 (41.0)
≥3		81 (19.9)	130 (29.6)

‡ P<0.05 farmers vs. controls.
† P<0.05 farmers with a farm childhood vs. farmers without a farm childhood.
# A familial history of allergy was diagnosed if two or more people among siblings or parents had an allergic disease.
§ Calculated from job history and standardized to years of 52 weeks/year and 40 hours/week. Median (± inter quartile range).
* Only farmer subjects were asked how many siblings they had. 407 farmers without a farm childhood and 439 farmers with a farm childhood answered this question.

with cattle and pigs was most extensive in farming students who had been born on a farm, while controls had spent only limited time with farm animals.

Prevalences of positive SPT reactions are shown in Fig.1 for farmers who had (n=501) or had not (n=487) lived on a farm during childhood, and for the whole group of conscripts (n=399). For practically all allergens the prevalence of sensitization was markedly lower for farmers than for controls, and - within the group of farmers - lower for those who had lived on a farm during childhood than for those who had not been a farm child. Interestingly, even sensitization to typical 'farm-related' allergens like storage mites and hair, danders or urinary proteins from farm-animals showed a lower prevalence among farmers than among controls. As a result, the prevalence of a positive SPT to 'any common allergen' ('SPT(5)') was significantly lower in conscripts, and - within farmers - significantly lower in those who had lived on a farm during childhood when compared to those who had not (Table 2).

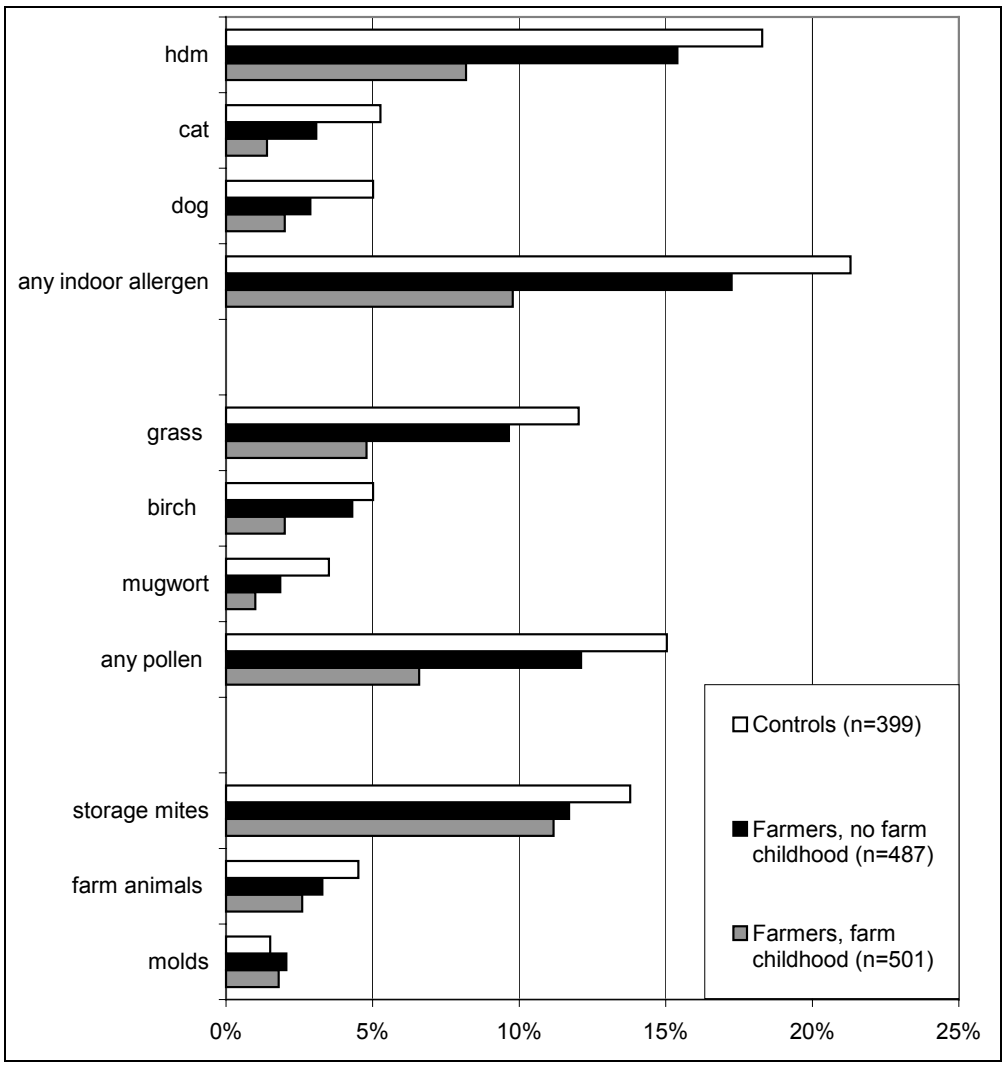

Figure 1. Sensitization against common and work-related allergens in young male adults in a rural environment. Prevalences of positive SPT in rural controls, farmers not born and raised on a farm, and farmers born and raised on a farm.

Wheeze was reported significantly less often by farmers than controls, but prevalences of asthma, rhinoconjunctivitis and BHR were similar (Table 2). However, within the group of farmers prevalences of respiratory symptoms and BHR were much lower in those who had lived on a farm during childhood when compared to those without a farm childhood. Prevalences of wheeze and asthma were therefore lowest in farmers with a farm childhood when compared to both conscripts and farmers without a farm childhood, while prevalences of rhinoconjunctivitis and BHR were highest in farmers without a farm childhood.

Table 2. Common atopy and respiratory health in study population according to current working life as a farmer and living at a farm in childhood.

	Controls*	Farmers, no farm childhood*	Farmers, farm childhood*
SPT(5)#	108/399 (27%) ‡	110/487 (23%) †	67/501 (13%)
Wheeze	50/402 (12%) ‡	53/494 (11%) †	27/505 (5%)
Asthma	33/395 (8%)	43/492 (9%) †	26/499 (5%)
Rhinoconjunctivitis	53/402 (13%)	78/494 (16%)	59/505 (12%)
BHR	31/380 (8%)	54/490 (11%) †	32/497 (6%)

‡ P<0.05 farmers vs. controls.
† P<0.05 farmers with a farm childhood vs. farmers without a farm childhood.
* Results are given as the number of subjects with a given characteristic (n) divided by the number of subjects for which the information was available (N).
# Positive SPT to one of the five common allergens house dust mite, grass pollen, birch pollen, cat or dog.

Table 3. Common atopy as defined by serology in restricted study population according to current working life as a farmer and living at a farm in childhood.

	Controls*	Farmers, no farm childhood*	Farmers, farm childhood*
IgE(5)#	101/141 (72%) ‡	99/161 (61%)	71/137(52%)
High total IgE§	83/143 (58%)	94/164 (57%) †	60/138 (43%)
Total IgE GM (±GSD)	110 (±4.0)	109 (±5.3)	78 (±4.9)

‡ P<0.05 farmers vs. controls.
† P<0.05 farmers with a farm childhood vs. farmers without a farm childhood.
* Results are given as the number of subjects with a given characteristic (n) divided by the number of subjects for which the information was available (N), except where indicated.
# Positive IgE test to one of the five common allergens house dust mite, grass pollen, birch pollen, cat or dog.
§ Serum IgE level of >100 kU/L.

Conscripts were shown as one group since only a small proportion (44 out of 402) had lived on a farm during childhood. Prevalences of sensitization in this subgroup were in general similar to those found in farmers without a farm childhood history: e.g. a positive SPT to 'any common allergen' ('SPT(5)') was found in 10 (22.7%) of the 44 conscripts with a farm childhood (not shown), compared to 110 (22.6%) of 487 farmers with no farm childhood (Table 2). In contrast, prevalences of respiratory symptoms and BHR were very similar for conscripts who had or had not lived on a farm during childhood.

There were no differences in smoking behavior, age, and working experience with cattle between the selected sample in which specific and total IgE was measured and the rest of the

Table 4. Present working life as a farmer and living at a farm in childhood as determinants of common atopy in young male adults in a rural environment. Multiple (logistic) regression analyses of the relation of both determinants with three atopy parameters, with adjustment for smoking habits and a familial history of allergy.

Atopy parameter	Determinant	OR	95% confidence interval	P
SPT(5) *	Farmer vs. control	0.75	[0.55 - 1.01]	<0.06
(n=1387)	Farm childhood	0.55	[0.40 - 0.76]	<0.001
	(Ever)smoking	0.97	[0.73 - 1.28]	>0.8
	Familial history of allergy	3.16	[2.02 - 4.93]	<0.001
IgE(5) #	Farmer vs. control	0.62	[0.39 - 0.98]	<0.05
(n=439)	Farm childhood	0.71	[0.46 - 1.10]	>0.1
	(Ever)smoking	0.81	[0.53 - 1.22]	>0.3
	Familial history of allergy	1.75	[0.87 - 3.52]	>0.1
High total IgE§	Farmer vs. control	0.90	[0.58 - 1.39]	>0.6
(n=445)	Farm childhood	0.68	[0.44 - 1.04]	<0.08
	(Ever)smoking	0.84	[0.56 - 1.25]	>0.3
	Familial history of allergy	1.68	[0.90 - 3.16]	>0.1

* Positive SPT to one of the five common allergens house dust mite, grass pollen, birch pollen, cat or dog.
\# Positive IgE test to one of the five common allergens house dust mite, grass pollen, birch pollen, cat or dog.
§ Serum IgE level of >100 kU/L.

population. However, a familial history of allergy was more common, the average number of siblings lower, and working experience with pigs less extensive in the former group (not shown). Because subjects with positive SPT responses were over-represented in this sample, frequencies of positive IgE EIA tests and total IgE levels were high (Table 3). Association of positive IgE tests with farm childhood and being a farming student however, showed a very similar pattern to that for positive SPTs. Relations for the various individual allergens were also similar, although no clear differences between groups were found for storage mite IgE sensitization.

A high concentration of total IgE (i.e., >100 kU/L) was strongly associated with positive SPT reactions (OR [95%CI] = 4.0 [2.6–6.2]) and with demonstrable IgE antibodies to common allergens (OR [95%CI] = 7.4 [4.7–11.8]). Levels of total IgE were significantly lower in farmers who had lived on a farm during childhood both compared to farmers who had not

Table 5. Present working life as a farmer and living at a farm in childhood as determinants of respiratory health status in young male adults in a rural environment. Multiple logistic regression analyses of the relation of both determinants with respiratory symptoms and BHR, with adjustment for smoking habits and a familial history of allergy.

Symptom	Determinant	OR	95% confidence interval	P
Wheeze	Farmer vs. control	0.69	[0.46 - 1.05]	<0.09
(n=1401)	Farm childhood	0.68	[0.43 - 1.06]	<0.1
	(Ever)smoking	3.41	[2.33 - 4.99]	<0.001
	Familial history of allergy	2.66	[1.50 - 4.71]	<0.001
Asthma	Farmer vs. control	0.92	[0.57 - 1.47]	>0.7
(n=1386)	Farm childhood	0.79	[0.49 - 1.27]	>0.3
	(Ever)smoking	2.43	[1.60 - 3.68]	<0.001
	Familial history of allergy	3.18	[1.75 - 5.77]	<0.001
Rhino-conjunctivitis	Farmer vs. control	1.24	[0.86 - 1.79]	>0.2
(n=1401)	Farm childhood	0.70	[0.49 - 0.99]	<0.05
	(Ever)smoking	1.04	[0.75 - 1.44]	>0.8
	Familial history of allergy	2.83	[1.74 - 4.59]	<0.001
BHR	Farmer vs. control	1.28	[0.82 - 2.02]	>0.2
(n=1367)	Farm childhood	0.61	[0.39 - 0.95]	<0.03
	(Ever)smoking	1.34	[0.90 - 1.98]	>0.1
	Familial history of allergy	1.39	[0.70 - 2.78]	>0.3

and compared to rural controls (Table 3). The latter two groups showed no difference, however.

In univariate analyses smoking, a familial history of allergy, the number of siblings, and working experience with cattle and pigs were all significantly associated with atopy, symptoms, or BHR (not shown). However, only smoking and a familial history of allergy were included in further multiple regression analyses in which the combined effects of being a farmer and having a farm childhood were assessed. Information on the number of siblings was available only for farming students. Adjusting for the number of siblings had no effect on the association between a farm childhood and prevalences of symptoms or BHR when analyses were restricted to farmers, and did only slightly weaken the association with the prevalence of SPT(5) (not shown). Working experience with pig and cattle was highly

associated with being a farmer and having had a farm childhood. When included in one model, the strength of associations between the latter parameters and wheeze, SPT(5) and IgE(5) slightly decreased, while associations with rhinoconjunctivitis became stronger (not shown).

Results of these regression analyses confirmed that both being a farmer, and a farm childhood contributed significantly to a lower risk of atopy defined either as at least one positive SPT ('SPT(5)') or as at least one positive specific IgE test ('IgE(5)'). For 'high total IgE', the OR for a farm childhood was below 1, but only borderline significant, while the OR for being a farmer was much less pronounced (Table 4). Also, in classical regression analysis only a weak, and not statistically significant, inverse relation was found between (log-transformed) levels of total IgE and being a farmer or having had a farm childhood (not shown). For respiratory health parameters, a negative association was found between prevalences of wheeze, asthma, rhinoconjunctivitis and BHR and a farm childhood, although this was significant only for the latter two. Current working live as a farmer showed only non-significant trends towards lower risks for wheeze and asthma and higher risks for rhinoconjunctivitis and BHR (Table 5).

Discussion

Our findings confirm and extend the results of other recent studies [2-4], which all showed that atopy is markedly less prevalent among children living or raised on a farm [1]. Among farming school students and conscripts from the same rural areas as controls, the prevalence of atopic sensitization to common allergens – assessed by SPT and/or IgE serology – was lowest in farmers who also in childhood had lived on a farm, intermediate in farmers without a farm childhood and controls with a farm childhood, and highest in controls without a farm childhood. Total serum IgE level was also lowest in farmers with a farm childhood, but was not significantly different between controls and farmers without a farm childhood. Prevalences of atopic symptoms in the whole group of farmers were not consistently different from those in non-farming rural controls, but were significantly lower in farmers with a farm childhood, compared to farmers without.

Several questions can be raised regarding the validity of the results. Investigation of the here-described relations was not the primary objective of the SUS project [13,14] and the design of the study might therefore not have been completely adequate. The question regarding the childhood home environment was added to the questionnaire after data on approximately

800 farming students had already been collected, and part of the original study population could thus not be included in our analyses. We further excluded all female farming students and all subjects aged over 26 to obtain a satisfactory match with the controls. Prevalences of positive SPTs, respiratory symptoms and BHR in the non-included groups were however similar to those in the analyzed population, and it therefore seems unlikely that a non-optimal design could have seriously biased the observed relations between atopy and childhood home environment.

Information on several potential confounders like antibiotic use, socio-economic status, attending day-care and number of siblings, was not or only partly available. Adjusting for such confounders changed the effect estimates for several atopic symptom parameters in some of the other studies in farm children [1-3,5], but had almost no effect on estimated ORs for atopic sensitization [1,3,5]. In our study, adjusting for the number of siblings within the group of farmers only slightly changed the estimated ORs for atopy in relation to a farm childhood (not shown). We therefore conclude that the 'protective' effect of a farm childhood against atopic sensitization and related respiratory symptoms can also be found in Denmark.

While most of the earlier studies investigated atopy in primary school children, our data show that the 'anti-atopy effect' of a farm childhood can still be found in young adulthood, also within a population of which currently all subjects are exposed to the farm environment. The Finnish study [4] investigated university students of nearly the same age as our farmers and controls and the results also suggested that the farm childhood effect can still be observed in young adulthood. However, since atopy was defined as self-reported atopic disease during any period in life, the reported low ORs might have been due mainly to associations with a history of atopy, and not with current atopic illness. Our data refer to the current or recent health status, and also found similar relations with SPT or serologic results, and thus confirm more definitely that the farm childhood-related anti-atopy effect would continue into adulthood.

Another new finding might be the association of a present working life as a farmer with a lower prevalence of atopy, independent of a farm childhood history. Many earlier studies have noticed low risks of typical type I allergy among farmers, either apparent from the relatively low prevalences of hay fever and asthma-like symptoms, or as relatively low prevalences of atopic sensitization to common allergens [16-19]. This low prevalence might be explained by a strong healthy worker effect, with atopic teenagers being less likely to start a

farming career with its known risks of high dust and allergen exposures, as suggested earlier [20]. The low prevalence of a familial history of atopic disease in farming students compared to controls (Table 1) may be regarded as evidence that at least part of the lower risk of atopy in farmers would be due to such pre-job selection. On the other hand, healthy worker selection would be driven primarily by (a history of) experienced allergic symptoms, and not directly by atopic sensitization – as long as the subject does not know his/her SPT or serologic results. In our study, negative associations between 'being a farmer' and symptoms were hardly found (Tables 2,5) and were much less pronounced than those with SPT or IgE test results. Thus, although a 'healthy worker' effect for respiratory symptoms or BHR can not be ruled out, the data suggest that also recent and current exposure of young adults to a farm environment may contribute to a lower risk of atopic sensitization to common aeroallergens.

A clear distinction between 'atopic sensitization' and 'atopic symptoms' or 'atopic disease' may also explain why – according to some critical comments [20] - the effect of a farm childhood is most pronounced with regard to hay fever and pollen sensitization, and much less to asthma, wheezing, and indoor allergen sensitization. In our experience and that of many others, self-reported hay fever in population studies shows a very strong association with positive pollen-specific SPT or IgE tests, while associations of self-reported wheezing and 'asthma' with indoor allergen sensitization are - although highly significant – in general much weaker. Apparently, much of self-reported wheezing and 'asthma' is of a non-atopic nature, and thus a relatively poor indicator of atopic sensitization compared to hay fever. In fact, the studies that included SPT or IgE serology have, just as our own study, found that a farm childhood is particularly associated with a low prevalence of atopic sensitization, not only to pollen but just as much also for indoor allergens [1,3], while relations with reported symptoms show less consistent patterns.

Given the wide spread nature of atopic diseases, it is of great importance to identify the farm environment-associated factors that would account for 'protection against atopy'. A genetic healthy farmer selection over a number of generations is highly unlikely [3], and thus external exposure factors must play a key role. Stimulation of the developing immune system towards Th1-dominated responses by microbial agents like endotoxins has been proposed as a plausible explanatory mechanism [21-23]. A recent report by Gereda et al. indeed showed a negative association between house dust endotoxin levels and the prevalence of atopic sensitization in young children in a non-farm community [24], and increased levels of

endotoxin in house dust from farmers' homes – particularly on farms with livestock like pigs, cows and horses -, have been reported [25]. If the presumed immune modulation of atopic responsiveness occurs via systemic mechanisms, exposure to endotoxins or similar agents via the gastro-intestinal tract (GIT) may however be important as well, and a different diet of farm children, e.g. including frequent consumption of non-pasteurized milk with enhanced levels of (other) microbial proinflammatory or immune-modifying agents, would be another plausible explanation [21,23,26-28]. On the other hand, if modulation of atopic immune responses to aeroallergens occurs primarily at the respiratory mucosae, exposure to airborne microbial agents would be more relevant. Thus, further epidemiological and experimental studies are required to identify the most relevant environmental or dietary factors that would account for the much lower prevalence of atopy in farming communities. Elucidation of the underlying mechanisms will be of major importance for a better understanding of the relations between changing environmental and dietary exposures and the risks of atopic sensitization and disease.

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Chapter 3

Endotoxin Exposure and Atopic Sensitization in Adult Pig Farmers

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ABSTRACT

Background Recent studies have reported a considerable lower prevalence of atopic sensitization and symptoms of respiratory allergy in children growing up on farms when compared to their peers living in the same rural areas.

Objectives Evaluate whether adult farmers also have a reduced risk for atopic sensitization and whether this is associated with endotoxin exposure.

Methods Complete data on endotoxin exposure, total serum IgE, and presence of IgE to HDM, grass pollen, birch pollen, and cat dander were available for 162 pig farmers from a cross-sectional case-control study. Exposure to endotoxin was measured on more than one occasion and modeled in detail. Exploratory analysis was done using non-parametric modeling and followed by classical multiple logistic regression.

Results IgE to one or more common allergens was detected in sera from 28 pig farmers (17%) and average (GM (GSD)) total serum IgE was 37 (4) IU/mL. A strong inverse relation was found between endotoxin exposure and sensitization to common allergens for exposures up to 75 ng/m3, with an OR [95%CI] of 0.03 [0.0 – 0.34] for a 2-fold increase in endotoxin. For endotoxin exposure above 75 ng/m3 the association was weak and not statistically significant (OR [95%CI] = 1.2 [0.38 – 3.6]). No association was found between endotoxin exposure and total serum IgE.

Conclusions The prevalence of atopic sensitization to common allergens in adult pig farmers is low. Exposure to endotoxin or related exposures appear to protect from sensitization to common allergens in an adult working population exposed to high levels of endotoxin.

J Allergy Clin Immunol. Provisionally accepted for publication.

Introduction

Recent epidemiological studies from several countries in Europe, Australia, and North America have reported a considerable lower prevalence of atopic sensitization and symptoms of respiratory allergy in children, young adolescents, and even adults growing up on farms when compared to their peers living in the same rural areas (for references see Johnson[1]). Contact with livestock during the first year of life was identified as the factor that best explained the protective effect on atopic sensitization,[2, 3] which is consistent with the view that early life is a critical period for the initiation of allergic immune responses and asthma.[4] Even though more specific protective factors were not determined in these studies, it has been speculated that respiratory exposure to endotoxin (particularly in livestock farming) may play an important role.[5-7] From studies in the work environment it is well known that animal keeping is associated with exposure to high levels of bacterial endotoxin.[8-10] Although there are strong indications that endotoxin exposure causes or aggravates (allergic) respiratory symptoms in farmers[11] and other exposed workers[12-14], experimental studies have shown that timing may be crucial. Consequently, studies on the possible "protective effect" of endotoxin exposure have focused almost exclusively on early childhood exposure and even prenatal exposure through the mother is considered as potentially relevant.[15-20]

Some studies have indicated that rural living[21] or being a farmer[22] is associated with a reduced risk of atopy and atopic disease in adulthood as well, and we have shown earlier that both having been brought up on a farm and current farming were independently associated with a lower prevalence of atopic sensitization in a study in farming students and conscripts from the same general areas as controls.[23] Only one study has investigated the separate effects of having been born on a farm and current endotoxin exposure.[16] Having been born on a farm was associated with a reduced risk of sensitization in school-age children, but there was an independent additional protective effect of current endotoxin exposure.

It is not clear whether adult farmers have a reduced risk for atopic sensitization and whether such a possible reduced risk is associated with endotoxin exposure. We therefore analyzed data from a study in pig farmers. Exposure to endotoxin was measured on more than one occasion and used to model long-term average exposure. Earlier analyses have shown that high endotoxin exposure was associated with respiratory symptoms and bronchial hyper-responsiveness, especially in atopic farmers.[24] The relation between endotoxin exposure and atopy was not analyzed in detail, although it was apparent that the prevalence of atopic

sensitization to common allergens in the population was low. We therefore investigated the relation between exposure to endotoxin and atopic sensitization in this cohort.

Methods

Population and health data

Data were from a cross-sectional survey in pig farmers conducted in the early 90s. A detailed description of the design and methods of data collection have been reported previously.[24, 25]

The population consisted of 194 pig farmers, living in the two South-Eastern provinces of the Netherlands, which were selected from a group of 1133 male owners of pig farms who worked at least five hours per day in pig farming. Selection was based on chronic respiratory symptoms reported in the Dutch version of a self-administered shortened questionnaire on respiratory symptoms of the British Medical Research Council.[26] All farmers (n=94) with more than one symptom of chronic cough, chronic phlegm, ever or, frequent wheezing, shortness of breath, and chest tightness (asthma) were included and a group of 100 controls was selected at random from the symptom free farmers. In a subsequent medical survey held in winter 1990/1991 venous blood samples were taken for IgE analysis. The medical ethical committee of the University Nijmegen approved the study. Subjects received information about the trial and consented to participate in writing.

IgE measurements

Sera were stored at -20°C until IgE analysis. Total IgE and specific IgE antibodies to the common allergens house dust mite, grass pollen (mix of two species), birch pollen, and cat were measured by enzyme immunoassays.[27]

Allergen-specific IgE was assessed with 1/10 diluted sera in allergen-coated microwells. A serum was considered positive if the optical density at 492 nm exceeded the mean optical density +3 SD of the reagent blank (no serum control). All positive sera were retested, all on the same day, together with an equal number of randomly selected negative sera. Only a small number of sera with a weakly positive reaction in the first test were eventually classified as negative, while all of the retested negative serum-allergen combinations remained negative in the second test.

Total serum IgE was measured by sandwich EIA, with sera diluted 1/10, 1/20 and 1/40, and as calibration standard the IgE standard for the Pharmacia CAP system.[27]

Exposure measurements and modeling

Personal inhalable dust samples were taken twice: one day in summer and one day in winter. Dust was collected using PAS6 sampling heads and 1 µm Teflon filters (Millipore) at an airflow of 2 L/min.[8] Samples were stored at –20 ºC until extraction. Endotoxin was extracted in 0.05% (v/v) Tween 20 in pyrogen-free water and stored at –20 ºC until analysis. [28] Endotoxin levels were measured using the Lymulus Amebocyte Test, according to procedures described earlier by Hollander *et al.*.[28] Endotoxin Units (EU) were converted to nanograms of endotoxin using a factor of 0.1 ng/EU.

Endotoxin levels were expressed in ng/m^3. Based on the relation between the endotoxin concentration and farm characteristics and time spent on activities in pig farming during two full weeks, the long-term average exposure to endotoxin was estimated.[29] The final model included outdoor temperature, 12 farm characteristics and 8 activities in pig farming and explained 37% (adjusted R^2 = 33%) of the variation in log-transformed time-weighted average endotoxin exposure. In this way data on endotoxin exposure was available for 162 farmers. In this paper endotoxin exposure is defined as modeled long-term average exposure to endotoxin.

Data analysis

IgE sensitization to common allergens was defined as a positive reaction to one or more common allergens. Endotoxin exposure and total IgE levels were best described by a lognormal distribution. Total IgE levels were either log-transformed or dichotomized, using 100 IU/mL as a cut-off level. For exploratory analysis the relation between log-transformed endotoxin exposure and sensitization to common allergens or (log-transformed) total IgE was studied using generalized additive modeling (smoothing) with PROC GAM (SAS for Windows version 8.0; SAS Institute, Cary, NC). For dichotomous response variables a logistic model was used, and smoothed curves were computed using a logit-link function and transformed to prevalences by applying the inverse of the logit-function. The smoothness of the function was determined by generalized cross validation (method=GCV).

Based on the results from this analysis either dichotomized or log-transformed endotoxin exposure was used in exact multiple logistic regression (PROC LOGISTIC). A p-value of <0.05 was considered statistically significant.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Subject characteristics, atopic sensitization, and total IgE

Complete data on endotoxin exposure, personal characteristics, and serology was available for 81 cases and 81 controls. Basic characteristics of the study population stratified by case-control status are presented in table 1. Cases were somewhat older, smoked more often, and had been working with pigs for a longer time than controls.

Table 1. Subject characteristics. Cases reported at least 1 chronic respiratory symptom (cough, phlegm, shortness of breath, wheeze, or chest tightness).

	Controls n (%)/mean (sd)	Cases n (%)/mean (sd)
N	81	81
Age	36 (9)	40 (10)
Smoker	15 (19)	37 (46)
Former smoker	25 (31)	25 (31)
Years in pig farming	13 (8)	16 (8)
Endotoxin exposure (ng/m3) (GM [GSD])	105 [1.4]	103 [1.5]

Specific IgE to at least one of the four common allergens was detected in 28 farmers (17%) and was weakly associated with case-control status (OR [95%CI] = 1.4 [0.63 – 3.3], table 2). Most sensitized farmers had IgE to HDM (61%) or grass pollen (36%), only 5 (18%) were sensitized to birch pollen and none were sensitized to cat allergens. 4 subjects (14%) were sensitized to more than one allergen.

Average (GM (GSD)) total serum IgE was 37 (4) IU/mL and was slightly higher in cases than in controls (table 2).

Table 2. Sensitization to common allergens and total serum IgE in pig farmers with and without chronic respiratory symptoms.

	Controls n (%)/mean (sd)	Cases n (%)/mean (sd)
N	81	81
IgE to common allergens		
≥ 1	12 (15)	16 (20)
house dust mite	6 (7)	11 (14)
grass pollen	6 (7)	4 (5)
birch pollen	3 (4)	2 (2)
cat dander	0 (0)	0 (0)
Total IgE ≥ 100 IU/mL	17 (21)	25 (31)
Total IgE (GM [GSD])	31 [4.0]	45 [3.9]

Endotoxin exposure and allergic sensitization

Endotoxin exposure was similar for cases and controls and ranged from 36-316 ng/m^3 (table 1). No association was found between high endotoxin exposure and specific sensitization (OR [95%CI] = 0.88 [0.34 – 2.3]) or raised levels (>100 IU/mL) of total IgE (OR [95%CI] = 1.2 [0.53 – 2.6]), when endotoxin exposure was dichotomized at the median level of 101 ng/m^3, as reported earlier.[24]

In contrast, the OR [95%CI] for a 2-fold increase in exposure and sensitization to common allergens was 0.44 [0.19 – 0.98] when log-transformed endotoxin exposure was included as a continuous covariate. Therefore, the shape of the exposure response relation was studied in more detail using non-parametric models (smoothing). Results from this analysis suggested a significant non-linear relation between exposure to endotoxin and sensitization to one ore more common allergen(s) (figure 1).

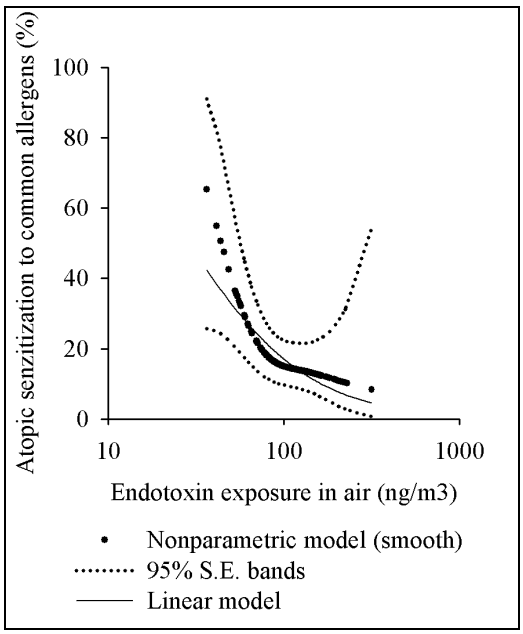

Figure 1. Inhalatory endotoxin exposure and IgE to common allergens in Dutch pig farmers. Linear model and smoothed (cubic spline) plot with point-wise ±2 SE bands.

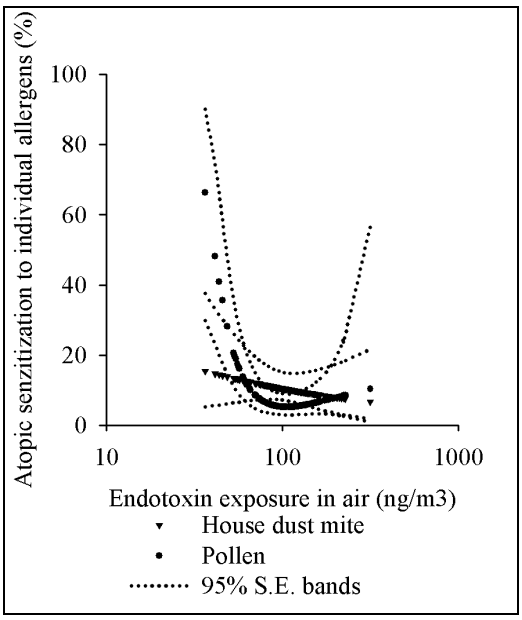

Figure 2. Inhalatory endotoxin exposure and IgE to pollen and house dust mite in Dutch pig farmers. Smoothed (cubic spline) plots with point-wise ±2 SE bands.

Risk of sensitization strongly declined with increasing exposure from a predicted 70% at the lowest exposure to 20% at an exposure of approximately 75 ng/m^3, and remained virtually unchanged for even higher exposures. We therefore allowed for different regression coefficients for endotoxin exposure below and above the level of 75 ng/m^3 in classical logistic regression analysis, including age, smoking habits, and case-control status as potential confounders. This 'broken-stick' model fitted the data significantly better than a model without break-point (Likelihood Ratio X^2 = 5.8; P=0.02) and the results confirmed the strong inverse relation between endotoxin exposure and sensitization to common allergens for exposures up to 75 ng/m^3 with an estimated OR [95%CI] of 0.03 [0.0 – 0.34] for a 2-fold increase in endotoxin. For endotoxin exposure above 75 ng/m3 the association was weak and not statistically significant (OR [95%CI] = 1.2 [0.38 – 3.6]). Inclusion of a familial history of atopy or disinfectant use as potential confounders had only minor effects on these estimates, and no evidence was found for effect modification by either case-control status or smoking habits in stratified analysis.

Inspection of the dose response curve for endotoxin exposure and sensitization to individual allergens suggested that the effect of endotoxin was strongest for sensitization to pollen, and

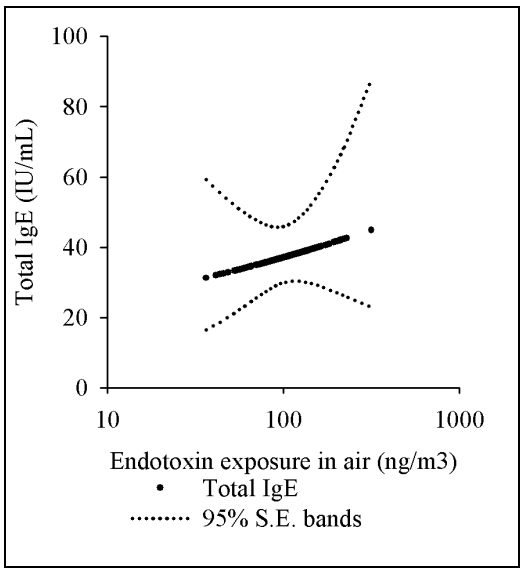

Figure 3. Inhalatory endotoxin exposure and total IgE in Dutch pig farmers. Smoothed (cubic spline) plots with point-wise ±2 SE bands.

that there was little effect on sensitization to house dust mite (figure 2). The small number of sensitized subjects did not allow any further detailed analysis.

The relation between endotoxin exposure and total IgE was also studied using non-parametric modeling (figure 3). Only a weak positive association was found between log-transformed endotoxin exposure and log-transformed total IgE levels (estimated coefficient β (SE) = 0.19 (0.30); P = 0.5), with no evidence of a non-linear relation. No association was found between endotoxin exposure and raised levels of total IgE (>100 IU/mL) in logistic regression.

Discussion

Only 17% of the full-time pig farmers in this study were sensitized to one or more common allergens, which almost certainly overestimates the population prevalence, as farmers with symptoms of respiratory disease were over-represented. Even so, it is considerably lower than the prevalence of 32% reported for males aged 20-70 years in the general Dutch population[30] and also much lower than prevalences of 27%-38% reported from occupational studies where the same IgE assays were used in bakers[31], workers in the potato processing industry[32], and laboratory animal workers.[33] Exposure to endotoxin was associated with a strongly reduced risk of sensitization for exposures up to 75 ng/m^3.

Together these results suggest that work-related exposure to endotoxin (or associated factors) rather than lifestyle factors explain the different prevalence of atopic sensitization in farmers and non-farmers, and that exposure to endotoxin protects from atopic sensitization even in adults. Severe confounding by differences in lifestyle factors is unlikely in this study as all subjects in the study were full-time pig farmers. The correlation between life style factors and occupational endotoxin exposure is therefore expected to be low. In addition, the large pig farms in the Netherlands cannot be compared to the small farms in Austria and Switzerland

where most research on farm children has taken place. Dutch pig farmers use modern technology to control environmental conditions and feeding rates and have a lifestyle that is probably much less traditional than that of their Mid-European colleagues. Confounding by childhood farm exposures is also unlikely as a possible explanation, as almost all farmers will have been born on a farm and the correlation between occupational endotoxin exposure and early life exposures is therefore probably very low. Information regarding childhood exposure was not available for this cohort, but more than 95% of pig farmers participating in a recent study in the same regions indicated they had been born on a farm (not published).

Although life style factors can most likely be ruled out, endotoxin might still be a surrogate marker for exposure to other agents of microbial or animal origin. The most important determinants of modeled exposure were activity patterns involving (close) contact with animals and flooring characteristics,[29] and these are probably not specific for endotoxin. Another question that this study is not able to answer is whether the lower prevalence of atopy is a consequence of a reduced incidence or an increased remission of sensitization. The fact that an effect was seen of *current* endotoxin exposure on sensitization to common allergens in *adults* suggests the latter process is involved.

Although there have been experimental studies on the effect of single endotoxin exposures on the initiation and polarization of allergic immune responses,[34-38] there is little information on the effect of prolonged endotoxin exposure on established immune responses.[39] Recently interest has moved beyond the classic Th1/Th2 paradigm and has focused on the role of regulatory T-cells in regulating immune responses to pathogenic and non-pathogenic agents. It is now thought that allergy is the result of a defect in this regulatory system and that high-dose exposure to microbial agents could lead to the induction of regulatory cells.[40, 41] This would seem to allow for a protective effect of endotoxin exposure after sensitization has occurred.

For individual allergens, endotoxin had a stronger effect on sensitization to grass pollen than on sensitization to HDM. Although the small number of sensitized subjects does not allow any strong conclusions, studies in farm children and farming adults have also pointed towards a possible differential effect of farming or endotoxin on indoor and outdoor allergens,[22, 42-45] which suggests the same causal mechanism might be involved. That we did not find an association between endotoxin exposure and total IgE levels could be considered inconsistent with an effect on atopic sensitization. However, others have argued that total IgE levels are more strongly hereditary, and less influenced by environmental factors.[46]

Major limitations of this study are the small population size and the fact that the study was designed to identify risk factors for chronic respiratory disease. Endotoxin exposure could be modeled for only 162 farmers and halve of these had been selected on the basis of rather general, but not work-related, respiratory symptoms. However, there was no evidence that the design had a major impact on the results, as the relation between endotoxin exposure and sensitization was similar for cases and controls. The allergen panel we used to define atopic sensitization did not include dog allergens. This is unlikely to have affected our results as sensitization to dog allergens is generally even less common than sensitization to cat allergens and we did not find a single farmer with IgE to cat allergens.

The assay that was used was based on an in house protocol. However, it had previously been validated against skin prick test results in a series of 116 children's serum samples.[27] Sensitivity and specificity of the EIA results as a predictor of skin prick test reactivity towards common allergens were >80%-90%. The assay has also been used extensively in other epidemiological studies.[31, 32, 47, 48]

Misclassification of exposure or IgE status could have occurred, but has probably been non-differential and would have led to underestimation of the exposure response relation. One of the goals of exposure modeling was to reduce misclassification by increasing the number of repeated exposure estimates per individual, as pig farmers usually stick to a weekly working schedule with considerable variation between days of the week.

Healthy worker selection could be a more serious problem as the relation between endotoxin exposure and sensitization would be seriously biased when those with allergic symptoms (and IgE) avoid exposure to endotoxin. However, only one subject reporting (non-work) related allergic symptoms was exposed at endotoxin levels < 75 ng/m^3 and a strong relation between endotoxin and sensitization was also found in those not reporting any symptoms.

Confounding by age, smoking habits, and case/control status has been taken into account in the present analysis, but other potential confounders like familial history of atopy, history of allergic symptoms in childhood, use of disinfectants, and number of years working with pigs were all considered. From the difference between our and earlier reported findings[24] it is clear that the cut-off value that is chosen for the logistic regression analysis is important. Our cut-off of 75 ng/m^3 was based on the results of an exploratory non-parametric model that clearly indicated a non-linear relation between endotoxin exposure and sensitization. However, using simple logistic regression with log-transformed modeled endotoxin exposure as a continuous covariate also resulted in a very low estimated OR for endotoxin and sensitization to

common allergens. Therefore, the cut-off of 101 ng/m^3 that was chosen for the earlier analysis is clearly sub-optimal.

In conclusion, average long-term endotoxin exposure was associated with a reduced prevalence of sensitization to common allergens in a highly exposed adult farming population. This relation seemed stronger for sensitization to grass pollen than for sensitization to HDM. These findings suggest that current endotoxin exposure might also be associated with a reduced prevalence of atopic sensitization in subjects without childhood exposure to high levels of endotoxin. However, results obtained in this same population have shown a clear relation between endotoxin exposure and presence of respiratory symptoms, decreased lung function, and increased lung function decline.[11, 24, 25] The potential beneficial effect of endotoxin on allergic respiratory disease therefore seems to be outweighed by its pro-inflammatory properties. Longitudinal studies are needed to confirm whether endotoxin protects from new onset sensitization or whether it primarily reduces pre-existing sensitization and to find out whether protection from sensitization has an effect on the development or severity of respiratory symptoms.

Acknowledgements

This work is supported by grant no. 99.33 from the Dutch Asthma Foundation (L. Portengen). We thank Professor James A. Deddens of the Department of Mathematics, University of Cincinnati, for his advice on evaluating non-linear dose-response relations.

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Chapter 4

Disinfectant Use and Respiratory Disease in Pig Farmers

ABSTRACT

Background An association between disinfectant use and atopic sensitization to common allergens and self-reported respiratory symptoms has been reported in pig farmers. We sought to study the association between disinfectant use and allergic sensitization, markers of airway inflammation, respiratory symptoms, and lung function in this population.

Methods Subjects filled out a questionnaire on disinfectant use, farm characteristics, job history, and respiratory symptoms. IgE antibodies to common allergens, pig urine, chloramine-T, and QAC's, and total IgE were determined in serum. Lung function was assessed by full forced spirometry and NO measured off-line in exhaled breath. Total and differential cell counts and the inflammatory cytokines IL1β, IL6, and IL8 were determined in nasal lavage fluid.

Results A total of 1079 farmers were invited to participate, and 133 (12%) attended the medical examination. 101 farmers worked with pigs for at least 24 hrs/week. Only one farmer was sensitized to QAC's. Disinfectant use was associated with a higher prevalence of IgE sensitization to common allergens (PR [95%CI] = 2.5 [0.4-17] for disinfecting weekly vs. never), although not statistically significant. Disinfectant use was also associated with prevalence of respiratory symptoms in atopic farmers, but not with lung function or NO in exhaled breath. IL8, and positive NAL's for IL1β and IL6 showed a positive dose-response with disinfectant use in both atopic and non-atopic farmers. Participation in the study was extremely low, but there was no evidence of significant selection bias.

Conclusion Exposure to disinfectants seems to be a risk factor for atopic sensitization to common allergens in pig farmers, as observed earlier, while IgE mediated allergy to disinfectants appears to be rare. Frequent disinfectant use may induce respiratory symptoms in atopic farmers, and may stimulate upper airways inflammation.

Introduction

Several studies have reported a relation between pig farming or work in swine confinement buildings and a high prevalence of respiratory symptoms[1-11], airways inflammation[12-17], lowered lung function[18-20], and increased lung function decline.[21-23] The generally low prevalence of sensitization to pig allergens[24-28] and the weak association between sensitization to pig allergens or sensitization to common allergens ('atopy') and symptom prevalence in most[24, 29, 30], but not all[31], studies have focused attention on exposure to non-allergenic agents like organic dust, mycotoxins, endotoxin, ammonia, hydrogen sulfide, and disinfectants.

Several disinfectants have been identified as respiratory hazards, mostly in case studies or case series from outbreaks of respiratory symptoms or allergies in hospitals.32-36 Many disinfectants are known or suspected irritants, but IgE mediated reactions have also been described, e.g. for aldehydes[34, 37], quaternary ammonium compounds (QACs)[38], and chloramine T.[32, 39] The lack of survey data raises the question whether the prevalence of sensitization to disinfectants may be largely underestimated. Intriguingly, results from a case-control study in 164 Dutch pig farmers suggest that exposure to disinfectants might also indirectly affect the development of respiratory symptoms.[30] Atopic sensitization to at least one of four common allergens occurred significantly more often in farmers who used disinfectants containing quaternary ammonium compounds (QAC's) than in those who did not use disinfectants. In addition, both endotoxin exposure and disinfectant use were risk factors for the presence of chronic respiratory symptoms, but only in atopic farmers.[30] This may be due to a stimulating effect of disinfectant exposure on the production of IgE (adjuvant effect) and the enhanced non-specific bronchial reactivity of atopics to irritants, including disinfectants.

Most information regarding the pathophysiology that underlies respiratory symptoms in pig farmers has been obtained from studies on small numbers of pig farmers15, 17, 40 or healthy volunteers exposed to swine dust in swine confinement buildings.41-49 Although these studies have shown an association between work in swine confinement buildings and levels of several markers of airways inflammation, the association with respiratory symptoms was not addressed. We evaluated the potential role of disinfectant use in the development of respiratory symptoms in a sample of pig farmers. We assessed sensitization rates to the most commonly used disinfectants and studied the association between disinfectant use and sensitization to common and work-related allergens, self-reported chronic respiratory

symptoms, and lung function. In addition, we determined the level of several markers of airways inflammation in exhaled breath and nasal lavage fluid.

Methods

Population and health data

The population consisted of 101 male pig farmers who worked with pigs for at least 24 hours per week. A commercial database was used to select pig farmers working on a farm with at least 150 breeding pigs or 300 fattening pigs and living in an area (ZIP-code) with at least 1 farm per square kilometer in the 3 southeastern provinces of the Netherlands. These criteria were chosen so as to obtain a sample of full-time pig farmers living relatively close to each other to facilitate the medical examination.

One-thousand seventy-nine (1079) farmers were invited by mail to participate in a short medical examination and fill out a questionnaire containing questions on personal characteristics (smoking, date of birth), presence of doctor diagnosed chronic disease, basic farm characteristics, and disinfectant use. A reminder was mailed 2 weeks later and farmers who did not respond to either mailing were phoned and personally invited to participate. One hundred and sixty-seven farmers (176; 15%) responded to one of the mailings and 37 (22%) decided to participate. The most frequent reasons for not participating were that subjects had recently quit pig farming (n=41; 32%) or did not use disinfectants (n=26; 20%). An additional 133 farmers agreed to participate after being contacted by telephone, resulting in a total of 170 subjects available for the medical examination. Thirty-seven farmers (22%) did not attend the medical examination, either because they were on holiday or because they had urgent business on the day of the examination. Finally, of the 133 subjects who attended the medical examination 101 (76%) worked with pigs for at least 24 hours per week.

Questionnaire

The questionnaire was based on the Dutch version of a self-administered shortened questionnaire on respiratory symptoms of the British Medical Research Council[50], but also included questions on the presence of allergic and non-allergic respiratory and skin symptoms, smoking habits, farm characteristics, as well as details of the disinfection procedure.

Spirometry

Full forced spirometry was assessed using a pneumotachometer (Jaeger Toennies, Hoechberg, Germany). Measurements were performed according to the lung function protocol of the European Community for Steel and Coal. FEV1, FVC, MMEF, and PEF were expressed as percentage of age-, height-, weight-, and sex-specific reference values.[51]

Serology

Sera were stored at -20°C until IgE analysis. Presence of IgE antibodies to a mix of common inhalatory allergens was determined by Phadiatop (Pharmacia, Woerden, the Netherlands). Positive sera were tested for specific IgE to either birch pollen (*Betula verrucosa*, t3), dog dander (e5), cat dander (e1), house dust mite (*Dermatophagoides pteronyssinus*, d1), rye grass (*Lolium perenne*, g5), or timothy (*Phleum pratense*, g6) with the UniCAP IgE assay (Pharmacia, Woerden, the Netherlands). All sera were also screened for the presence of IgE antibodies to pig urinary proteins (Re212), suxamethonium (succinylcholine, Rc202; a QAC), and chloramine T (k85).

Total IgE was measured by a slightly modified enzyme immunoassay as reported before.[52] In short, microwells were coated overnight at 4°C with monoclonal mouse anti human IgE (M1294, Sanguin, the Netherlands) 1/4000 in PBS pH=7.0. Sera were diluted 1/5, 1/15, and 1/45 in PBS containing 0.05% v/v Tween-20 and milk proteins (PBTM), and incubated for one hour at 37°C. Binding of IgE was detected by incubation with rabbit anti human IgE (1/10000 in PBTM; A0094, DAKO) for one hour at 37°C, followed by incubation with peroxidase labeled swine anti rabbit IgG (1/8000; PO399, DAKO) for one hour at 37°C, and incubation with OPD for 30 minutes at 20°C. The enzyme reaction was ended by adding 50 µl 2 N HCl, and the optical density was read at 492nm. Reference preparations for total IgE (10-9123-01, Pharmacia, the Netherlands) were used to obtain a linear calibration line for concentrations from 0.5 to 5 IU/L (sample range 2.5 – 225 IU/L). Enhanced total IgE was defined as total IgE > 100 IU/mL.

Exhaled Breath

Subjects remained seated throughout the measurement and were instructed to take a deep breath through an NO scrubbing filter and exhale at a constant speed via a flow restrictor into a mylar balloon. All subjects filled 2 balloons. Up to three balloons were filled with ambient air at the start of the day, after approximately 3 hours, and at the end of the day (after approximately 6 hours). The NO concentration in the balloons was determined with a

chemiluminescence analyzer (Sievers 280i NOA, Boulder, CO, USA). All measurements were adjusted for the daily readings of mylar balloons filled with medical grade air (n=3) and a certified NO gas (65 ppb, n=3) respectively (both from Hoek Loos, Barendrecht, the Netherlands).

Nasal Lavage

Nasal lavage was performed as described previously.[53] Total cell count was determined in NAL fluid and cell differentials in a cytospin. IL1β, IL6, and IL8 were measured in NAL fluid by EIA (Biosource, Biosource Europe SA, Fleurus, Belgium). Limits of detection were 6.5 pg/ml, 8 pg/ml, and 3 pg/ml for IL1β, IL6, and IL8 respectively.

Data analysis

All statistical analyses were performed using SAS software (version 8.02; SAS Institute, Cary, N.C.). Most continuous variables followed an approximate lognormal distribution, and were therefore described by their geometric mean (GM) and geometric standard deviation (GSD) and log-transformed before use in regression analysis. Crude differences in prevalence rates or (geometric) means between groups were compared using Fisher's exact test and Student's t test. Prevalence Ratios and 95% confidence limits were calculated by log-binomial regression (PROC GENMOD). PROC GENMOD was also used to calculate geometric mean ratios (GMR) and corresponding confidence limits for predictors of log-transformed outcomes by exponentiating regression coefficients and confidence limits to allow interpretation on the original scale. All multiple linear or log-binomial regression analyses were adjusted for age and smoking habits except where indicated. Years in pig farming, weekly hours working with pigs, farm type, and several proxies for farm size were considered as potential confounders, but were not or only weakly associated with disinfectant use. Inclusion in the multiple regression models had only minor impact on the effect estimates for disinfectant use. All statistical tests were done 2-sided and a p-value <0.05 was considered significant.

Results

Subject characteristics and disinfectant use

Basic characteristics of the study population, frequency of disinfection, and type of disinfectant used are presented in table 1. Pig farmers using QAC's had been working with pigs for a shorter period (mean (SD) = 20 (7.8) years; n=62) than those using other

Table 1. Subject characteristics.

	n (%)/Mean (sd)
N	101
Age (years)	43 (8.8)
Standing height (cm)	179 (7.0)
Years in pig farming	21 (7.9)
Weekly hours working with pigs	55 (14.5)
Current smoker	19 (19%)
Former smoker	32 (32%)
Frequency of disinfection	
- Never	16 (16%)
- Less than once a week	37 (37%)
- At least once a week	47 (47%)
Type of disinfectant used	
- none	16 (16%)
- Chloramine-T, no QACs	17 (17%)
- QACs, no Chloramine-T	52 (51%)
- both Chloramine-T and QACs	10 (10%)
- other	6 (6%)

disinfectants (mean (SD) = 24 (6.9) years; n=23 $P<0.05$), but frequency of disinfection and type of disinfectant used were not significantly associated with other personal characteristics.

Sensitization to common and occupational allergens and total IgE

Sera were obtained from 95 full-time pig farmers and IgE antibodies to at least one of the common allergens were detected in sera from 15 (16%; table 2). Thirteen subjects had IgE to HDM, 7 to grass and/or tree pollen and 4 to pet dander. Only one subject was sensitized to pig urine, (another) one to QAC's, and none to chloramine T. Levels of total IgE above the detection limit of 2.5 IU/L were found in sera from 88 farmers (93%) and followed an approximate log-normal distribution with a GM (GSD) of 36 (6) IU/mL. Total IgE >100 IU/mL (enhanced total IgE) was found in sera from 25 (26%).

Table 2. Sensitization to common allergens and raised total IgE (n (%)) in pig farmers by frequency of disinfection and type of disinfectant used.

		Disinfectant Use					
	All	Never	<52/year	≥52/year	Chloramine-T	QAC	Both
N	95	16	33	46	13	51	9
IgE ≥1 common allergen	15 (16%)	1 (6%)	5 (15%)	9 (20%)	3 (23%)	9 (18%)	2 (22%)
Total IgE > 100 IU/mL	25 (26%)	3 (19%)	8 (24%)	14 (30%)	4 (31%)	15 (29%)	1 (11%)

Prevalence proportions of sensitization to common allergens and of enhanced total IgE tended to increase with increasing frequency of disinfection, but these trends were not statistically significant (table 2). For sensitization to common allergens adjusted PR's [95%CI] obtained in multiple log binomial regression analysis were 1.8 [0.2-13] for disinfecting less than weekly vs. never and 2.5 [0.4-17] for disinfecting at least weekly vs. never after adjusting for age and smoking habits. For enhanced total IgE the corresponding PR's [95%CI] were 1.2 [0.4-3.8] and 1.6 [0.5-4.8] respectively. Prevalence of sensitization or enhanced total IgE were not different for users of QAC's or chloramine T.

Respiratory symptoms

Prevalence of chronic respiratory symptoms in this population is summarized in table 3. Thirtysix (36%) full-time pig farmers reported one or more chronic respiratory symptoms, of which wheeze and phlegm were the most frequently reported. A breakdown by frequency of disinfection and use of specific disinfectants is also presented in table 3. No association was found between either of these exposure surrogates and presence of chronic respiratory symptoms.

Table 3. Respiratory symptoms (n (%)) in pig farmers by frequency of disinfection and type of disinfectant used.

		Disinfectant Use					
Symptoms*	All	Never	<52/year	≥52/year	Chloramine-T	QAC	Both
N	101	16	37	48	17	52	10
Cough	7	0	3	4	2	3	2
	(7%)	(0%)	(8%)	(8%)	(12%)	(6%)	(20%)
Phlegm	13	3	5	5	2	7	1
	(13%)	(19%)	(14%)	(10%)	(12%)	(13%)	(10%)
Shortness of Breath	5	1	2	2	1	2	1
	(5%)	(6%)	(5%)	(4%)	(6%)	(4%)	(10%)
Wheeze	25	4	7	14	5	14	2
	(25%)	(25%)	(19%)	(29%)	(29%)	(27%)	(20%)
Chest Tightness	8	1	3	4	3	2	2
	(8%)	(6%)	(8%)	(8%)	(18%)	(4%)	(20%)
Any	36	6	12	18	8	19	3
	(36%)	(38%)	(32%)	(38%)	(47%)	(37%)	(30%)

* Cough: almost daily during at least 3 months in past 2 years.
Phlegm: almost daily during at least 3 months in past 2 years.
Shortness of breath: when walking with people of similar age at an ordinary pace on the level.
Wheeze: ever.
Chest Tightness: ever.

Sensitization to common allergens was a risk factor for presence of respiratory symptoms (PR [95%CI] = 2.0 [1.1-3.6], after adjusting for age and smoking habits), and this relation was particularly strong for wheeze (PR [95%CI] = 2.7 [1.2-6.0]) and chest tightness (asthma, PR [95%CI] = 6.0 [1.5-25]). Disinfectant use appeared to be a risk factor for presence of respiratory symptoms in atopic farmers, as 7 out of 9 farmers (78%) sensitized to common allergens and disinfecting at least weekly reported chronic respiratory symptoms, but only 2 out of 6 atopic farmers (33%) disinfecting less frequently (or not at all). However, the low number of sensitized farmers did not allow formal statistical evaluation of this possible modifying effect of atopic sensitization. For farmers with enhanced total IgE the proportion of farmers with respiratory symptoms was similar for those disinfecting at least weekly (7/14=50%) and those disinfecting less often (5/8=63%), although it was lowest in farmers who did not use any disinfectants (1/3=33%). These relations were also not statistically significant.

Lung function

Lung function data for the study population are presented in table 4. Average lung function was higher then predicted based on reference equations obtained in a general population sample.[51] No association was found between frequency of disinfection or type of disinfectant used and lung function. There was no evidence of effect modification by the presence of atopy or enhanced total IgE.

Table 4. Lung function in pig farmers by frequency of disinfection and type of disinfectant used. TIFF is the ratio of FEV1 to FVC.

		Disinfectant Use					
	All	Never	<52/year	≥52/year	Chloramine-T	QAC	Both
N	101	16	37	48	17	52	10
FEV1 (L)	4.1 (0.7)	4.0 (0.9)	4.1 (0.7)	4.1 (0.7)	4.0 (0.8)	4.2 (0.7)	3.8 (0.7)
FVC (L)	5.4 (0.8)	5.4 (0.8)	5.5 (0.8)	5.4 (0.8)	5.4 (0.9)	5.5 (0.8)	5.1 (0.7)
TIFF (%)	76 (8)	73 (11)	76 (8)	77 (8)	75 (9)	78 (7)	75 (9)

Inflammatory markers in exhaled breath and NAL

Exhaled breath was obtained from all 101 subjects, and NO concentrations followed an approximate lognormal distribution with a GM (GSD) of 18 (1.6) ppb. No association was found between frequency of disinfection or type of disinfectant used and NO in exhaled breath.

Ninety-three full-time farmers participated in nasal lavage. Mean volume (SD) of recovered nasal lavage fluid was 6.1 (2.0) mL, with a total cell count (GM (GSD)) of 1708 (4.9) cells/mL. IL8 could be detected in all samples (GM (GSD) = 243 (1.9) pg/ml), but levels of IL1β and IL6 were below the limit of detection in the majority of samples. All 6 samples with detectable levels of IL1β also had detectable levels of IL6, and IL8 was on average 70% higher in farmers with detectable levels of IL1β/IL6 (GMR [95%CI] = 1.7 [1.1 – 2.7]; P = 0.02).

Sixteen cytospin preparations were discarded as they were of poor quality (n=7) or did not have enough cells to allow a differential count (n=9). The majority of cells in the 77 remaining preparations were either neutrophils (mean (SD) = 48% (30)) or epithelial cells (mean (SD) = 43% (32)). Mononuclear cells were present in small numbers (GM (GSD) = 5.8% (3.0)). Eosinophils were found in only a minority of samples (n (%) = 33 (43%), and ranged from 0.5% - 12%.

Descriptive statistics for the levels of inflammatory markers in exhaled breath and NAL fluid, along with a breakdown by disinfectant use, are presented in table 5. Levels of inflammatory cytokines in the NAL fluid and presence of eosinophils in cytospin preparations increased with increasing frequency of disinfection, although this was statistically significant only for IL8. Geometric mean ratios [95%CI] for the relation between disinfection frequency and IL8 obtained in multiple regression analysis were 1.5 [1.0-2.2] for disinfecting less than weekly vs. never and 1.8 [1.2-2.6] for disinfecting at least weekly vs. never after adjusting for age and smoking habits. No association was found between frequency of disinfection and total number of cells and cell type frequencies in NAL fluid.

The potential relevance of the association between disinfection frequency and levels of inflammatory markers in exhaled breath or NAL fluid was further investigated by studying the relation between these markers and presence of respiratory symptoms or sensitization to common allergens (table 6). Presence of respiratory symptoms was associated with presence of IL1β, IL6, and eosinophils in NAL fluid, but not with levels of NO in exhaled breath or levels of IL8 and total cell numbers in NAL fluid. Atopic sensitization to common allergens was associated with increased levels of NO in exhaled breath and detectable IL1β, IL6, and eosinophils in NAL fluid.

Table 5. Presence of inflammatory markers in exhaled breath and nasal lavage fluid in full-time pig farmers by frequency of disinfection and type of disinfectant used. NO was measured in exhaled breath and expressed as geometric mean (GSD). Cytokines were determined in nasal lavage fluid presented as the number of samples (%) with levels above the detection limit (for IL1β and IL6) or as geometric mean levels (GSD) in pg/ml (for IL8). Cells were counted in nasal lavage fluid and total cell count is expressed as a geometric mean (GSD) in absolute numbers/ml. Differential counts were performed in cytospin preparations. Presence of eosinophils is expressed as a simple dichotomy (yes/no), neutrophils as a mean (SD) percentage of total cells and mononuclear cells as geometric mean (GSD) percentage of total cells in the lavage fluid.

		Disinfectant Use					
	All	never	<52/year	>52/year	Chloramine T	QAC	Both
N (Exhaled breath)	101	16	37	48	17	52	10
NO (ppb)	18 (1.6)	18 (1.7)	18 (1.5)	18 (1.6)	16 (1.7)	19 (1.6)	18 (1.5)
N (NAL)	93	13	34	46	14	51	10
IL1ß (≥detection limit)	6 (6%)	0 (0%)	2 (6%)	4 (9%)	1 (7%)	5 (10%)	0 (0%)
IL6 (≥detection limit)	8 (9%)	0 (0%)	3 (9%)	5 (11%)	1 (7%)	7 (14%)	0 (0%)
IL8 (pg/ml)	243 (1.9)	159 (2.1)	236 (2.0)*	280 (1.7)**	236 (1.5)	272 (1.9)**	245 (2.0)
Total cells (cells/ml)	1708 (4.9)	1692 (6.2)	1930 (6.4)	1564 (3.7)	1375 (3.3)	2018 (5.0)	1302 (7.2)
N (cytospins)	77	9	30	38	12	43	8
Eosinophils (≥detection limit)	33 (43%)	2 (22%)	14 (47%)	17 (45%)	7 (58%)	18 (42%)	3 (38%)
Neutrophils (% of cells)	48 (30)	42 (34)	50 (29)	47 (30)	46 (28)	50 (31)	50 (20)
Mononuclear cells (% of cells)	5.8 (3.0)	6.4 (3.8)	4.8 (2.8)	6.5 (3.0)	8.0 (3.0)	5.6 (2.8)	5.3 (4.1)

* $P < 0.05$ compared to farmers who did not use disinfectants

** $P < 0.01$ compared to farmers who did not use disinfectants

Table 6. Presence of inflammatory markers in exhaled breath and nasal lavage fluid in pig farmers and associations with respiratory symptoms and sensitization status. NO was measured in exhaled breath and expressed as geometric mean (GSD). Cytokines were determined in nasal lavage fluid presented as the number of samples (%) with levels above the detection limit (for IL1β and IL6) or as geometric mean levels (GSD) in pg/ml (for IL8). Cells were counted in nasal lavage fluid and total cell count is expressed as a geometric mean (GSD) in absolute numbers/ml. Differential counts were performed in cytospin preparations. Presence of eosinophils is expressed as a simple dichotomy (yes/no), neutrophils as a mean (SD) percentage of total cells and mononuclear cells as geometric mean (GSD) percentage of total cells in the lavage fluid.

		Respiratory symptoms		GMR/PR/ß	Sensitized to common allergens		GMR/PR/ß
	All	None	Any	[95%CI]	None	Any	[95%CI]
N (Exhaled breath)	101	65	35		80	15	
NO (ppb)	18 (1.6)	18 (1.5)	18 (1.7)	1.0 [0.8 - 1.2]	17 (1.5)	22 (2.1)	1.3 [1.0 - 1.7]*
N (NAL)	93	61	32		74	14	
IL1ß (≥detection limit)	6 (6%)	2 (3%)	4 (13%)	3.7 [0.7 - 20]	4 (5%)	1 (7%)	2.1 [0.2 - 19]
IL6 (≥detection limit)	8 (9%)	3 (5%)	5 (16%)	2.9 [0.7 - 12]	4 (5%)	3 (21%)	4.5 [1.0 - 20]*
IL8 (pg/ml)	243 (1.9)	233 (1.9)	263 (1.9)	1.1 [0.8 - 1.5]	243 (1.9)	233 (1.7)	1.0 [0.6 - 1.4]
Total cells (cells/ml)	1708 (4.9)	1674 (4.9)	1774 (5.0)	1.1 [0.5 - 2.2]	1717 (5.1)	1367 (5.0)	1.0 [0.4 - 2.6]
N (cytospins)	77	53	24		62	10	
Eosinophils (≥detection limit)	33 (43%)	20 (38%)	13 (54%)	1.3 [0.8 - 2.2]	24 (39%)	7 (70%)	2.3 [1.4 - 3.7]*
Neutrophils (% of cells)	48 (30)	44 (32)	56 (25)	11 [-3 - 27]	47 (30)	57 (26)	15 [-6 - 37]
Mononuclear cells (% of cells)	5.8 (3.0)	5.5 (3.3)	6.4 (2.3)	1.1 [0.6 - 1.9]	5.5 (3.1)	8.5 (2.0)	1.4 (0.6 - 3.0)

* $P < 0.05$

¶ β and 95% confidence interval [95%CI] from multiple linear (log binomial) regression models adjusting for age and smoking habits. β's and confidence limits exponentiated for log binomial regression models and linear models with log-transformed outcomes.

Discussion

These results confirm earlier findings that disinfectant use is associated with a higher prevalence of atopic sensitization to common allergens in pig farmers.[30] However, the overall prevalences of IgE sensitization to common and work-related allergens in this and the earlier study among pig farmers were low. Less than 20% appeared to be sensitized to any of five common allergens, compared to prevalence proportions of approximately 30% reported for sensitization to any of four common allergens in surveys in Dutch and other West-European populations.[54, 55] This is in accordance with other studies that have shown a low prevalence of sensitization against common and farm-related allergens in farmers and farm children.[56-63]

Despite the presumably often-repeated exposure to high levels of disinfectants, only one worker showed demonstrable IgE sensitization to QAC's. No clear evidence was found that disinfectant use might be associated with the presence of respiratory symptoms or lower lung function. Among farmers who were sensitized to common allergens however, those who disinfected at least weekly reported respiratory symptoms more often than those who did not. Interestingly, a stronger relation between disinfectant use and respiratory symptoms in atopic farmers was also found by Preller et al. [30], who also found an association between disinfectant use and IgE sensitization and respiratory symptoms in pig farmers. Unfortunately, our study lacked statistical power to make a proper assessment of this relation, as the number of sensitized farmers was small.

We performed NAL to assess signs of upper airway inflammation. Given the generally high background level of airways inflammation in this population due to high endotoxin exposure[42, 44], an effect of disinfectant exposure on levels of inflammatory markers in NAL fluid would be difficult to detect. Nevertheless the results from the nasal lavage suggested that frequent exposure to disinfectants might lead to increased production of inflammatory cytokines in the upper airways. Use of disinfectants was also associated with a higher prevalence of positive tests for IL1b and IL6 in NAL fluids and more samples with detectable eosinophils, while a positive dose-response was found between disinfection frequency and levels of IL8.

These increased cytokine levels would not be the consequence of acute airway inflammation, but may rather reflect a more chronically increased 'sub-clinical' or 'background' mucosal inflammation, as NALs were obtained at the medical examination, and not directly or shortly

after exposure to disinfectants at work. Moreover, the association remained when farmers who had disinfected on the day of the medical examination or the day before were excluded from the analysis (not shown). Other studies have also reported higher levels of inflammatory cytokines in NAL fluids from compost workers that were obtained before work on a Monday morning when compared to those from unexposed controls.[53] This suggests that cytokine levels in the upper airways may remain higher for some days after exposure has ceased and may not only reflect acute inflammatory responses.

The relevance of these associations between disinfectant use and cytokine levels for the development of respiratory health effects is unclear. Levels of IL8 in NAL were not associated with presence of respiratory symptoms or sensitization to common allergens and IL1b and IL6 could be detected in only a very small number of NAL samples.

A major drawback of the study was the relatively small size, due to a low response rate among the farmers. Our study population was selected from a database of approximately 1,100 addresses and phone numbers of pig farmers, and only 12% were willing to participate. The power of the study to detect associations between disinfectant use and health parameters was therefore limited, and self-selection may have occurred that could have biased exposure response relations. The low participation rate could largely be ascribed to the outbreak of Classical Swine Fever in the Netherlands in 1997/1998. This stimulated legislature aimed at reducing the size of the pig herd (Pig Farming Restructuring Act; 1998) and relocating remaining farms in so-called concentration areas (Reconstruction Bill; 1999). To obtain a sample of full-time pig farmers only, farmers working on a farm with at least 150 breeding pigs or 300 fattening pigs had been selected from the database. However, when contacted, many farmers had recently quit farming or had switched to raising chickens or goats. Many others expressed growing frustration with government regulations and were generally reluctant to participate.

Looking for evidence of possible self-selection, we compared some general demographic characteristics of our study population with e.g. those from farmers participating in the European Union Concerted Action "Prevalence and risk factors for airway obstruction in farmers"[10] and other studies.[1, 5, 14, 18, 19, 64, 65] The prevalence of respiratory symptoms in our study seemed to be a little lower than in most of the other studies, but this could perhaps be explained by a cohort effect as smoking rates in our population were also much lower. Compared to the most recent large Dutch pig farmers study,[7] overall symptom prevalence

was rather similar, although prevalence of chronic cough (and smoking) was lower in our study.

Exposure to disinfectants was based on questionnaire responses, which might have been subject to non-differential misclassification. However, there were not enough subjects to allow a more refined analysis. Any bias resulting from this type of misclassification will generally lead to lower effect estimates, although this may not be true for all separate categories when exposure is classified in more than two categories. Years in pig farming, weekly hours working with pigs, farm type, and several proxies for farm size were all considered as potential confounding variables, but could not explain the results we obtained (not shown). However, farmers not using disinfectants may run their farm differently than those that do in other aspects as well, and residual confounding can therefore not be completely excluded.

Exposure to disinfectants seems to be a risk factor for allergic sensitization to common allergens in this population and may also lead to an increase in the prevalence of respiratory symptoms in atopic farmers. Disinfectant use was not associated with presence of respiratory symptoms in non-atopic farmers. Prevalence of positive samples for IL1b and IL6 and levels of IL8 in NAL fluids showed a positive dose-response with disinfection frequency, and this relation was present also in farmers who had not recently used disinfectants, suggesting that non-acute effects may (also) be responsible.

As the power of this study was low, many of the estimates are rather imprecise, and a larger and longitudinal study would be preferable. However, recruitment and continued participation of a large group of pig farmers does not seem feasible at present, and we do not know of other occupational groups that are similarly high exposed.

Acknowledgements

This work is supported by grant no. 99.33 from the Dutch Asthma Foundation. The authors wish to thank all farmers for participating in this study and Siegfried de Wind, Anoek Besselink, Anjoeka Pronk, and Jacques Spithoven for their assistance in data collection and laboratory analysis.

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Chapter 5

Disinfectant Use and Respiratory Allergy in Laboratory Animal Workers

ABSTRACT

Background Disinfectant use in pig farmers has previously been shown to be a risk factor for atopic sensitization to common allergens, self-reported respiratory symptoms, and accelerated lung function decline. It is unknown whether these relations also exist in other populations, or whether disinfectant use may also be associated with sensitization to work-related allergens or respiratory disease. We studied the association between disinfectant use and sensitization to common and laboratory animal (LA) allergens, self-reported respiratory symptoms, and lung function in LA workers known to be exposed to highly potent rodent allergens.

Methods Data were from a large longitudinal study in LA workers. Information on health outcomes was obtained during the first survey, while disinfectant use was estimated from data collected one year later. Workers were classified either as not using disinfectants or only alcohol, using chloramine-T, or using other disinfectants. Data were analyzed separately for workers who had been working with LA's for more than 4 years ('experienced' workers) or less than 4 years ('novice' workers).

Results Information on disinfectant use was available for 311 'experienced' and 193 'novice' workers. No association was found between the type of disinfectant used and allergic sensitization or respiratory health in 'experienced' workers. Among 'novice' workers prevalence of sensitization to common allergens (Prevalence Ratio (PR) [95%CI] = 0.6 [0.3-1.0]) and sensitization to LA allergens (PR [95%CI] = 0.3 [0.1-0.9]) were both lower in workers using chloramine-T compared to those not using disinfectants or only alcohol. Prevalence of self-reported symptoms of respiratory allergy to common allergens (PR [95%CI] = 0.4 [0.2-0.8]) and LA's (PR [95%CI] = 0.5 [0.3-1.0]) were both lower in workers using disinfectants. A strong inverse relation was also found between disinfectant use and self-reported allergic respiratory symptoms that started before the age of 17 (PR [95%CI] = 0.2 [0.1-0.7]).

Conclusion A higher risk of allergic sensitization to common allergens associated with disinfectant use, as reported in pig farmers, could not be confirmed in LA workers. Disinfectant use was associated with a lower prevalence of allergic sensitization and self-reported allergic respiratory symptoms in 'novice' LA workers. Potential explanations for these different findings include the use of different disinfectants, the probably much lower exposure levels in laboratory animal workers, and reversed causation due to exposure avoidance by allergic workers. A role for disinfectant use as a strong risk factor for respiratory allergies or other respiratory health effects in this population seems unlikely.

Introduction

Several disinfectants have been identified as respiratory hazards, mostly in individual cases or case series from outbreaks of respiratory symptoms or allergies in hospitals.[1-5] Many disinfectants are known or suspected irritants, and IgE mediated allergic reactions have also been described e.g. for aldehydes[3, 6], quaternary ammonium compounds (QACs)[4, 7], and chloramine-T.[1, 8] Results from a case-control study in 198 Dutch pig farmers suggested that disinfectant use might also indirectly induce respiratory symptoms by stimulating the production of IgE antibodies to common allergens. Farmers using disinfectants (particularly those containing quaternary ammonium compounds (QAC's)) were more often sensitized to common allergens than farmers who did not.[9] In addition, disinfectant use was associated with self-reported chronic respiratory symptoms in atopic farmers and with accelerated lung function decline.[10] Prevalence of sensitization to work-related allergens was very low in this population, and an effect on work-related IgE sensitization or respiratory disease could therefore not be determined.

We wanted to evaluate the possible role of disinfectant use in the development of respiratory symptoms in an occupational group where IgE-mediated allergies are much more common. Laboratory animal workers are exposed to highly potent rodent allergens, and prevalence of IgE-mediated respiratory allergy to animal allergens in these workers is high and estimated variously between 14-44%.[11]

Data were available from a large longitudinal study in laboratory animal workers conducted in the Netherlands, consisting of three annual cross-sectional surveys during the period 1992 - 1995. We studied the association between disinfectant use and IgE sensitization to common and laboratory animal allergens, self-reported respiratory symptoms, and lung function.

Materials and Methods

Study design and subjects

Data were from a cross-sectional survey conducted in 1992/1993, and repeated in 1993/1994 and 1994/1995. The design and methods of data collection have been described in detail previously.[12] Each survey included a self-administered questionnaire, blood samples, a lung function test, and skin prick tests (SPTs) with laboratory animal allergens. SPTs with common allergens were performed only at the first survey; SPTs with laboratory animal

allergens were not repeated when there had been a strong reaction to one of these allergens at the first survey.

Data were analyzed separately for workers who had been working with laboratory animals for more than 4 years ('experienced' workers) or less than 4 years ('novice' workers), since earlier reports on this population showed evidence of healthy worker selection in workers with longer job histories.[12, 13] The cut-off point was chosen because many 'novice' workers were Ph.D. students, who had an employment contract period of 4 years.

Questionnaire

The main questionnaire contained questions on respiratory symptoms, symptoms of allergy attributed to working with laboratory animals, smoking history, contact with laboratory animals, and occupational history.[14] The questionnaire used at the first survey contained additional questions on symptoms of allergy attributed to common allergens and a familial history of allergy. When subjects reported symptoms of allergy attributed to common allergens they were asked to indicate at what age symptoms had started and, if applicable, at what age these had ceased.

Individual work histories were recorded at each visit and subjects were asked how long they had been working with laboratory animals prior to enrolment in the study. Workers were asked to estimate the number of hours they had been working with laboratory animal species, faeces, or urine during an appropriate time interval in the year before the visit.

A parental history of allergy was defined as allergic asthma, hay fever, or eczema in at least one of the parents. Work-related respiratory symptoms were defined as self-reported chest tightness, sneezing, or a runny nose attributed to working with rats, mice, rabbits, or guinea pigs.

Skin prick testing and total IgE

Skin prick testing was performed as described previously[12] with five common allergens (mixture of *Dermatophagoides pteronyssinus* and *D. farinae*; mixture of grass pollen; mixture of tree pollen; cat fur; dog fur, all from ALK Benelux, Houten, The Netherlands), six laboratory animal allergen preparations (rat urine; rat fur; mouse urine; mouse fur; rabbit fur; guinea pig fur, all from ALK Benelux) and positive (histamine) and negative (PBS) controls. A wheal diameter of 3 mm or more, after subtraction of the response to the negative control, was regarded as a positive skin prick test (SPT). Atopy was defined as a positive SPT to at least

one of the common allergens. Laboratory animal sensitization was defined as a positive SPT to at least one of the laboratory animal allergens.

Sera were stored at -20°C until IgE analysis. Total serum IgE was measured with a sandwich enzyme immunoassay in sera obtained during the first survey as detailed in Doekes *et al.* (1996).[15] Enhanced total IgE was defined as serum IgE above 100 kU/L.

Spirometry

FVC, FEV_1, and MMEF were recorded with a Vicatest V dry rolling seal spirometer (Jaeger, Breda, The Netherlands). Measurements were performed according to the lung function protocol of the European Community for Steel and Coal[16]. Lung function change was defined as the difference in lung function between the third and first survey divided by the time interval between these surveys. For this analysis only lung function data from the first and last survey were used, as change in lung function over a shorter period may be strongly influenced by short-term variability leading to loss of power.[17]

Exposure assessment

Information on disinfectant use was collected at the second and third survey. Subjects were asked what type of disinfectant they used and how often. Information on frequency of disinfectant use was missing for most workers, and subjects were therefore classified as either not using disinfectants or using only alcohol, using chloramine-T, or using other disinfectants.

Complete information on respiratory health outcomes and results from skin prick tests were available from first survey data and disinfectant use at the second survey was therefore used as a proxy for exposure during employment.

Statistical analysis

Crude differences in prevalence rates between groups were compared using Chi square tests, while t-tests and F-tests were used to compare the means of continuous variables. Adjusted prevalence ratios (PR) were calculated by generalized linear modeling using a log link and assuming a binomial error distribution.[18] All multiple regression analyses included sex, age, smoking habits, parental history of allergy, and weekly hours worked with laboratory animals during the year before the first survey as potential confounding variables. All statistical analyses were performed using SAS software (version 8.02; SAS Institute, Cary, N.C.). Statistical tests were done 2-sided and a p-value <0.05 was considered significant.

Table 1. Subject characteristics. Workers were classified as 'experienced' when they had been working with laboratory animals for more than 4 years prior to the first survey.

	'Experienced' workers n (%)/mean (SD)	'Novice' workers n (%)/mean (SD)
N	311	193
Sex (female)	88 (28%)	110 (57%)*
Age (years)	39 (8.9)	28 (6.2)*
Standing height (cm) §	176 (8.9)	175 (9.4)
Years working with laboratory animals (yrs)	15.6 (9.0)	2.0 (1.3)*
Hours worked with laboratory animals during year preceding the first survey (hrs/yr) #	509 (570)	357 (449)*
Current smoker	79 (25%)	40 (21%)
Former smoker	109 (35%)	30 (16%)*
Parental history of allergy #	102 (33%)	78 (41%)
Type of disinfectant used at 2nd survey		
- None & alcohol	161 (52%)	127 (66%)
- Chloramine-T & other	61 (20%)	34 (18%)
- Other, no Chloramine-T	89 (29%)	32 (17%)*

* $P < 0.05$
§ information missing for 1 'experienced' worker
# information missing for 1 'novice' worker

Results

Subject characteristics and disinfectant use

Basic descriptive characteristics of the study population are presented in table 1. Of 504 subjects with complete questionnaire data, 193 (38%) had been working with laboratory animals for less than 4 years.

Average (SD) interval between the first and second survey was 1.0 (0.16) year. Two hundred and seventy-five workers (55%) reported using disinfectants at the second survey. Use of alcohol (n=124, 25%) and chloramine-T (n=95, 19%) were reported most frequently, while other disinfectants were used by only few subjects (<5%). No subjects reported using QAC's. Fifty-nine workers (12%) used alcohol only and these were pooled with subjects not using

disinfectants to gain statistical power, as a significant effect of inhalatory alcohol exposure on sensitization or respiratory health outcomes was not expected. Results were very similar when these workers were either excluded from the analysis or included as a separate exposure category.

Classification according to disinfectant use did not change for at least 65% of subjects in any of the three categories when reported disinfectant use at the *third* survey was used instead, indicating that disinfectant use remained fairly stable over a 1-year period (Cohen's Kappa [95%CI] = 0.55 [0.47-0.62]; n=400).

Laboratory animal workers using chloramine-T and/or other disinfectants were slightly older (mean [SD] = 36 [10.8] vs. 34 [8.5] yrs; P<0.01), worked with animals much more frequently (mean [SD] = 600 [616] vs. 329 [428] hrs/yr; P<0.01), (had) smoked more often (62% vs. 43%; P<0.01), and less often reported a parental history of allergy (31% vs. 39%; P=0.06) than those who did not. 'Novice' workers used disinfectants less often than 'experienced' workers (34% vs. 48%; P < 0.01), but the use of chloramine-T did not differ (18% vs. 20%; n.s.).

Sensitization to common and work-related allergens and total IgE

Prevalence of a positive SPT to common and laboratory animal allergens and enhanced total IgE are presented in table 2, along with a breakdown by type of disinfectant used. Two hundred and thirteen (213; 46%) laboratory animal workers were sensitized to one or more common allergens, 130 (28%) were sensitized to one ore more laboratory animal allergens, and 83 (18%) had enhanced total IgE. Prevalence proportions of positive SPT's and enhanced total IgE were similar for 'novice' and 'experienced' workers.

No association was found between the type of disinfectant used and results of SPT's or presence of enhanced total IgE in 'experienced' workers. In 'novice' workers, subjects using chloramine-T appeared to have a lower prevalence of allergic sensitization as assessed by SPT, compared to workers not using disinfectants or using only alcohol. This was confirmed in multiple regression models, adjusting for sex, age, smoking habits, parental history of allergy, and hours worked with laboratory animals during the year before the first survey as potential confounders. Prevalence ratio's (PRs) [95%CI] for using chloramine-T and sensitization to common and laboratory animal allergens in 'novice' workers in these models were 0.6 [0.3-1.0] and 0.3 [0.1-0.9] respectively.

Table 2. Sensitization to common and laboratory animal allergens and elevated total IgE in 'experienced' and 'novice' laboratory animal workers by type of disinfectant used.

		Disinfectant		
Outcome #	All	None/Alcohol	Chloramine-T	Other
'Experienced' workers	289	151	57	81
SPT+ ≥1 common allergen	134 (46%)	70 (46%)	31 (54%)	33 (41%)
Total IgE > 100 IU/mL	49 (17%)	22 (15%)	10 (18%)	17 (21%)
SPT+ ≥1 laboratory animal allergen	86 (30%)	43 (28%)	18 (32%)	25 (31%)
'Novice' workers	177	114	34	29
SPT+ ≥1 common allergen	79 (45%)	53 (46%)	10 (29%)	16 (55%)
Total IgE > 100 IU/mL	34 (19%)	22 (19%)	5 (15%)	7 (24%)
SPT+ ≥1 laboratory animal allergen	44 (25%)	32 (28%)	4 (12%)*	8 (28%)

For timing of skin prick testing and collection of serum in relation to estimated exposure see methods section.
§ SPT = Skin Prick Test
* $P < 0.05$ compared to those not using disinfectants or only using alcohol

Respiratory symptoms

Prevalence of chronic and allergic respiratory symptoms in this population and a breakdown by type of disinfectant used are presented in table 3. One hundred and fifty subjects (150; 30%) reported chronic respiratory symptoms, 102 (20%) complained of respiratory symptoms after contact with common allergens (dust, animals, or pollen), and 96 (19%) reported respiratory symptoms after contact with laboratory animals (rats, mice, rabbits, or guinea pigs).

No relation was found between disinfectant use and presence of chronic respiratory symptoms in 'experienced' workers. There appeared to be a trend towards a lower prevalence of allergic respiratory symptoms in workers using disinfectants, but the effect was weak and not statistically significant. PRs [95%CI] for using any disinfectant other than alcohol versus not using disinfectants (or using alcohol only) and presence of self-reported respiratory symptoms attributed to contact with common allergens or laboratory animals were 0.8 [0.5-1.3] and 1.0 [0.6-1.5] respectively, after adjusting for potential confounders in multiple regression models.

Table 3. Chronic respiratory symptoms and respiratory symptoms attributed to contact with common allergens and laboratory animals in 'experienced' and 'novice' laboratory animal workers by type of disinfectant used.

		Disinfectant		
Outcome #	All	None/Alcohol	Chloramine-T	Other
'Experienced' workers	311	161	61	89
Chronic respiratory symptoms	92 (30%)	49 (30%)	19 (31%)	24 (27%)
Respiratory symptoms attributed to contact with common allergens	62 (20%)	37 (23%)	10 (16%)	15 (17%)
Respiratory symptoms attributed to contact with laboratory animals	63 (20%)	38 (24%)	9 (15%)	16 (18%)
'Novice' workers	193	127	34	32
Chronic respiratory symptoms	58 (30%)	43 (34%)	6 (18%)	9 (28%)
Respiratory symptoms attributed to contact with common allergens	40 (21%)	34 (27%)	3 (9%)*	3 (9%)*
Respiratory symptoms attributed to contact with laboratory animals	33 (17%)	27 (21%)	2 (6%)*	4 (13%)

For timing of collection of questionnaire data pertaining to symptoms in relation to estimated exposure see methods section.
* $P < 0.05$ compared to those not using disinfectants or using only alcohol

In 'novice' workers the relation between disinfectant use and presence of allergic respiratory symptoms was much stronger, and PRs [95%CI] for using any disinfectant other than alcohol versus not using disinfectants (or alcohol only) and presence of self-reported respiratory symptoms to common allergens or laboratory animals were 0.3 [0.1-0.7] and 0.5 [0.3-1.0] respectively. This relation could not be explained by the lower prevalence of IgE sensitization in workers using disinfectants, as estimates changed little when sensitization to common or laboratory animal allergens was included in the regression models.

To evaluate whether the inverse association between disinfectant use and respiratory symptoms to common allergens in 'novice' workers could be due to reversed causation, we investigated the association between disinfectant use and allergic respiratory symptoms to common allergens reported to have started before the age of 17. This age was chosen as any causal relation between our proxy for disinfectant exposure and respiratory symptoms before that age would be very unlikely. In 'novice' workers using no disinfectants or only alcohol 25 subjects (20%) reported having had allergic respiratory symptoms before the age of 17, while

Table 4. Lung function in 'experienced' and 'novice' laboratory animal workers by type of disinfectant used. Adjusted lung function values were obtained in multiple linear regression analysis, adjusting for sex, age, standing height, smoking habits, parental history of allergy, and hours worked with laboratory animals during the year before the survey.

		Disinfectant		
Outcome #	All	None/Alcohol	Chloramine-T	Other
'Experienced' workers (N)	306	157	61	88
FEV1 (L)	4.1 (0.8)	4.2 (0.8)	4.0 (0.9)	4.1 (0.8)
adjusted FEV1		4.1	4.0	4.1
FVC (L)	5.1 (1.0)	5.2 (1.1)	5.0 (1.0)	5.1 (1.0)
adjusted FVC		5.1	5.1	5.1
TIFF (%)§	80 (7.0)	81 (6.6)	79 (8.1)	81 (6.9)
adjusted TIFF		81	79	81
'Novice' workers (N)	186	121	34	31
FEV1 (L)	4.1 (0.8)	4.2 (0.8)	3.8 (0.7)*	3.9 (0.6)
adjusted FEV1		4.1	4.0	4.1
FVC (L)	5.0 (1.0)	5.1 (1.1)	4.7 (1.0)*	4.6 (0.8)*
adjusted FVC		5.0	4.9	4.9
TIFF (%)§	83 (6.2)	83 (5.9)	83 (6.9)	85 (6.2)*
adjusted TIFF		83	82	84

For timing of lung function testing in relation to estimated exposure see methods section.
§ ratio of FEV1 to FVC.
* $P < 0.05$ compared to those not using disinfectants or using only alcohol

this was true for only 4 subjects using chloramine-T or other disinfectants (6%; P=0.01). The association also remained in multiple regression analysis, with an estimated PR [95%CI] for disinfectant use of 0.2 [0.1-0.7].

Lung function

Average FEV_1, FVC, and TIFF, and a breakdown by type of disinfectant used are shown in table 4. Average lung function in the study population was somewhat higher than predicted based on age-, sex-, and height-dependent reference equations (FEV_1 as %predicted = 107%, FVC as %predicted = 111%; n=492).

There was no association between disinfectant use and lung function in 'experienced' workers, but lung function in 'novice' workers appeared to be somewhat lower in subjects using disinfectants. Average [SE] differences in FEV_1 and FVC between workers using disinfectants and those not using disinfectants (or using alcohol only) were -322 [118] mL and -463 [156] mL (both $P < 0.01$). Differences were much smaller and no longer statistically

significant in multiple regression analysis, adjusting for sex, age, smoking habits, standing height, and hours worked with laboratory animals during the year before the survey.

Mean [SD] annual changes in FEV_1 and FVC in 'novice' workers (n=115) were -19 [98] mL/yr and +5 [112] mL/yr, calculated over an average follow-up of 2.0 years. Decline in lung function was somewhat more pronounced in 'experienced' workers (n=217) with mean [SD] annual changes in FEV_1 and FVC of -54 [99] mL/yr and -26 [117] mL/yr. Mean annual change in lung function in workers using disinfectants was similar to that in workers not using disinfectants, and regression coefficients for disinfectant use obtained in multiple linear regression analysis were small and not statistically significant (not shown).

Discussion

A higher risk of allergic sensitization to common allergens associated with disinfectant use, as reported in pig farmers[9], could not be confirmed in this study among laboratory animal workers. No association was found between disinfectant use and sensitization or respiratory health outcomes in workers who had been working with laboratory animals for more than 4 years. In workers with shorter job histories disinfectant use was associated with a lower prevalence of allergic sensitization and self-reported allergic respiratory symptoms to both common and work-related allergens. A role for disinfectant use as a strong risk factor for respiratory allergies or other respiratory health effects in this population therefore seems unlikely.

Several factors may explain the different relations we found in this study compared to those in pig farmers, where disinfectant exposure was associated with a higher prevalence of atopic sensitization to common allergens and chronic respiratory symptoms, and accelerated lung function decline.[9, 10] First, associations found in that study were most pronounced for disinfectants containing QAC's, and these were not used by any of the workers in our study. Second, exposure to disinfectants was probably much lower than in pig farmers. Pig farmers sometimes used high-pressure sprayers to disinfect an entire piggery, and this was one of the factors associated with an accelerated lung function decline in that study.[9] Third, the association between IgE mediated sensitization and respiratory symptoms is probably much stronger than in pig farmers. However, this cannot explain the different associations between disinfectant use and IgE mediated sensitization that we found. Fourth, reversed causation may have biased our results. Many disinfectants (including chloramine-T) are known or suspected irritants that may trigger symptoms in allergic subjects[19], who may therefore avoid

using these agents. This would be less likely to occur in pig farmers as these are mostly self-employed and exposure can therefore not be avoided as easily. The fact that we had to estimate disinfectant use from questionnaire data obtained at a later survey also renders our study more vulnerable to this type of bias. An indication that reversed causation may explain our findings is the fact that laboratory animal workers using disinfectants less often reported a parental history of allergy and less often reported allergic respiratory symptoms before the age of 17 than those not using disinfectants. However, inclusion of a parental history of allergy as potential confounder had only minor effects on the results from the multiple regression analysis, while the inverse relation between disinfectant use and allergic respiratory symptoms before the age of 17 could also be due to differential recall of symptoms by currently symptomatic versus non-symptomatic workers. Also, complete reversal of the relation between exposure and health effect would require very strong selective pressures, that are unlikely to exist for exposure to disinfectants and allergic respiratory symptoms.

Finally, the relation between disinfectants and allergy could be confounded by job characteristics, as disinfectant use is likely to be associated with jobs requiring more frequent handling of laboratory animals. Although this would be expected to result in a positive association between disinfectant use and laboratory animal sensitization, other job characteristics and requirements may also be important. Among 'novice' workers, PhD students used disinfectants significantly less often than other workers, but had a higher prevalence of sensitization to laboratory animal allergens (not shown). This could possibly be explained by more intense pre-job selection of (sensitized) 'non-PhD' laboratory animal workers during vocational training. However, an inverse relation between disinfectant use and sensitization or symptomatic allergy was found also within 'PhD' and 'non-PhD' laboratory animal workers.

In conclusion, results from this study do not support a role for disinfectant use as a strong risk factor for sensitization and respiratory allergy in this population. Exposure to disinfectants was associated with a lower risk of allergic sensitization and a lower risk of respiratory symptoms to both common and laboratory animal allergens in laboratory animal workers with limited previous working experience. No association was found between disinfectant use and health effects in workers with longer job histories. A protective effect of disinfectant use on respiratory allergy cannot be excluded, but exposure avoidance by allergic workers and residual confounding by job type may be more plausible alternative explanations.

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Chapter 6

IgG4 Antibodies to Rat Urinary Allergens, Sensitization and Symptomatic Allergy in Laboratory Animal Workers

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ABSTRACT

Background and Objectives We have previously reported that high rat urinary allergen (RUA) exposure was not associated with increased risk of rat allergy in long-term exposed laboratory animal workers. We aimed to assess whether strong allergen-specific IgG4 responses could explain the absence of a dose response in these subjects. We investigated whether IgG4 was associated with allergen exposure and prevalence of sensitization or respiratory symptoms to rats. The longitudinal relation between IgG4 and rat allergy was studied using data obtained during two years of follow-up.

Methods Five hundred and twenty-nine laboratory animal workers answered a questionnaire on respiratory symptoms and occupational history and participated in skin prick testing. Blood samples were analyzed for specific IgG4 and IgE to RUA. Exposure to RUA was estimated based on personal air samples. The relation between IgG4 and newly occurring sensitization or rat allergy was studied in workers who were not sensitized or did not report respiratory symptoms to rats.

Results IgG4 titers were higher in atopic than in non-atopic subjects, and increased with higher allergen exposure. Titers were highest in subjects who were sensitized and reported respiratory symptoms to rats when compared to those who were not (GM [GSD]=202 [5.7] vs. 8.4 [18.3] AU). The association between IgG4 and sensitization or symptomatic rat allergy was independent of estimated allergen exposure. IgG4 was a strong predictor of newly occurring sensitization and symptomatic rat allergy during follow-up in atopic and rat-sensitized subjects.

Conclusion High exposure to RUA is associated with a strong allergen-specific IgG4 antibody response. High anti-RUA IgG4 is a strong predictor of prevalent and incident sensitization and symptomatic rat allergy in atopic and rat-sensitized subjects. IgG4 can therefore not explain the absence of a dose response between allergen exposure and allergy in long-term exposed workers. We consider anti-RUA IgG4 to be a marker that combines aspects of exposure and susceptibility.

Clin Exp Allergy 2004; 34:1243-50

Introduction

Several studies have investigated exposure response relationships for sensitization to common and occupational allergens in domestic and specific occupational environments (as reviewed by [1-3]). Sensitization occurs for different allergens at very different exposure levels indicating that considerable differences exist in sensitizing potential[3]. Few studies have evaluated the shape of the exposure response relationships for allergic sensitization in detail, but there is evidence from cross-sectional studies that for some allergens sensitization risk levels off at higher exposure levels or is even bell shaped[4, 5].

In a study among children aged 12-14, highest exposure to the major cat allergen Fel d 1 was associated with a lower prevalence of sensitization (as determined by RAST)[5]. High exposure to cat allergen was associated with an IgG antibody response in the absence of sensitization and with a low risk of asthma. IgG antibodies to Fel d 1 included a large proportion of IgG4, an isotype of which the production is dependent on IL4, and the authors therefore referred to this as a modified Th2 response. As those with the IgG(4) response were also not symptomatic, this was considered a form of high-dose tolerance. Others have suggested that bell shaped exposure response relationships may be the result of allergen exposure avoidance by subjects at high risk for allergies [4, 6].

From studies on occupational allergy it has long been known that the presence of detectable levels of allergen-specific IgG4 is more common in those with prolonged exposure to high doses of the allergen involved[7-9]. In these studies presence of IgG4 was not associated with symptomatic allergy, and was therefore regarded as a marker of exposure. Studies on desensitization immuno-therapy have shown that successful therapy is associated with the induction of high levels of allergen-specific IgG4, although this is now thought to reflect the induction of regulatory T-cells, rather than being a causal factor in the outcome[10].

We have previously reported that in laboratory animal workers a clear dose-response relation between allergen exposure and risk of sensitization and symptomatic rat allergy could only be found in workers who had been working with laboratory animals for a relative short period[11]. As sensitization and symptoms to rat allergens were less prevalent at high exposure levels among workers with long job histories, the absence of a dose response in those workers was attributed to healthy worker selection. However, alternative explanations, e.g. a form of high-dose tolerance in at least some of these workers, could not be ruled out.

In this paper we investigate whether the presence of high levels of allergen-specific IgG4 could be responsible for the absence of a dose response in workers with longer job histories in this cohort. We studied the relation between exposure to rat urinary allergens (RUA) and IgG4 response and compared IgG4 levels in allergic and non-allergic workers. We also investigated whether levels of IgG4 predict development of sensitization or allergic respiratory symptoms to rat allergens during follow-up.

Materials and Methods

Study design and subjects

Data were from a cross-sectional survey conducted in 1992/1993, and repeated in 1993/1994 and 1994/1995. Detailed description of the design and methods of data collection have been reported previously[11]. Each survey included a questionnaire and skin prick testing. A total of 586 workers participated in the baseline study and IgG4 to RUA was assessed in sera from 529 (90%). 453 and 381 subjects were seen again during the first and second follow-up respectively, 466 workers (88%) were seen at least once during follow-up. 63 workers were lost to follow-up either because they did not work anymore in the laboratory animal facility or because they had holidays or were ill during the field study periods.

Seventyseven (15%) reported respiratory symptoms (chest tightness, sneezing, or a runny nose) attributed to working with rats at the first survey, and of those without symptoms 399 (88%) were seen at least once during follow-up. Valid skin prick test data from the first survey were available for 470 workers, and 86 (18%) had a positive skin prick test to rat urine or rat hair. Of those with a negative skin prick test result 315 (82%) had valid skin prick tests from at least one follow-up survey. Follow-up cohorts for incident symptomatic allergy and sensitization therefore consisted of 399 and 315 subjects respectively.

Questionnaire

The questionnaire contained questions on respiratory symptoms, airway hyperresponsiveness, allergic symptoms due to common and occupational allergens, smoking history, contact with laboratory animals, and occupational history[12]. For his paper the following questionnaire items were used:

Chronic asthma was defined as the presence of symptoms of shortness of breath, wheeze (ever), or chest tightness, while chronic bronchitis was defined as the presence of symptoms

of cough or phlegm. Presence of symptoms of allergy to common allergens was defined on the basis of self-reported allergic symptoms (chest tightness, runny or sneezing nose, runny or itchy eyes, and itchy or red skin) to house dust, pollen, and/or domestic animals. Presence of symptoms of hyperresponsiveness was defined on the basis of self-reported difficulties in breathing when going from warm to cold surroundings, from cold to warm surroundings, during foggy weather and/or during cold weather. Symptomatic respiratory rat allergy was defined as the presence of self-reported respiratory symptoms (chest tightness, sneezing, or a runny nose) attributed to working with rats.

Exposure assessment

Individual work histories were recorded at each visit and subjects were asked how long they had been working with laboratory animals prior to enrolment in the study. Detailed information on the quantification of rat urinary allergens exposure can be found elsewhere[13]. Based on the information collected during the visits intensity of exposure (in three categories) was estimated for each subject[11]. Subjects were asked to estimate the number of hours they had worked with rats, faeces or urine during an appropriate interval in the year before the visit. Exposure to RUA was defined as the product of exposure intensity and the number of hours a subject had worked with rats or rat products during the year preceding the survey, as earlier cross-sectional analyses in this population had revealed that this predicted specific skin test sensitization best.

Based on the numbers of years a subject had been working with laboratory animals at the first survey, exposure duration was classified as either short-term (≤4 years) or long-term (>4 years) in keeping with earlier reports on this population [11].

Skin prick testing

Skin prick testing was performed as described previously [26] with five common allergens (mixture of *Dermatophagoides pteronyssinus* and *D. farinae*; mixture of grass pollen; mixture of tree pollen; cat fur; dog fur, all from ALK Benelux, Houten, The Netherlands), six occupational allergens (rat urine; rat fur; mouse urine; mouse fur; rabbit fur; guinea pig fur, all from ALK Benelux) and positive (histamine 10 mg/ml) and negative (PBS) controls. Common atopy was defined as a positive skin prick test (wheal size ≥ 3mm) to at least one common allergen. Sensitization to rats was defined as a positive skin prick test to rat urine or rat fur.

IgG4 serology

Sera were stored at -20°C until analysis. Specific IgG4 antibodies to RUA were determined by a modified EIA as described previously[8]. Microwells were coated overnight at 4°C with 200 μl of a 10 μg/mL preparation of rat urinary proteins in PBS (pH=7.0). Details of the allergen preparation have been described by Hollander *et al.*[13]. After blocking with PBS containing 0.05% v/v Tween-20 and 0.2% w/v gelatin (PBTG; pH=7.4), sera were tested at 1/2 in PBTG in duplicate. After incubation for 1 hour at 37°C binding of specific IgG4 was determined by incubation with peroxidase-labelled monoclonal mouse anti-human IgG4 (CLB, Amsterdam, the Netherlands) diluted 1:1000 in PBTG (1 hour at 37°C), followed by incubation for 30 minutes at 20°C with 2 mg/mL OPD (Sigma, St. Louis, USA) in citrate/phosphate buffer (pH = 5.5), containing 0.015% v/v hydrogen peroxide. The reaction was stopped by adding 50 μl of a 2M HCl solution and the optical density read on a spectrophotometer at a wavelength of 405nm. Antibody concentrations were expressed as relative titers, calculated by interpolation of the logit-transformed OD-values on a calibration line obtained in the same microtiterplate with a pool of strongly positive sera, which was arbitrarily given an antibody titer of 100. For samples with a logit value above +3, the analysis was repeated at higher dilutions (10-2000).

IgE serology

Specific IgE antibodies to RUA were measured by immunoassay (AlaSTAT; DPC, Apeldoorn, the Netherlands)[14].

Statistical analysis

Crude differences in prevalence rates between groups were compared using Fisher's exact test, while F tests were used to compare the means (or geometrical means) of continuous variables. Simple bivariate relations between 2 continuous variables were investigated by correlation analysis, using Spearman's rank correlation coefficient (ρ) whenever applicable.

Exposure to RUA and titers of IgE and IgG4 to RUA did not follow a normal distribution, and were log-transformed and presented as geometric mean (GM) and geometric standard deviation (GSD) where necessary. For subjects not working with rats exposure was arbitrarily set at two-thirds of the lowest calculated exposure level in those working with rats (1.18 ng/m3*hrs/yr) before log transformation. Titers of IgG4 below the detection limit were replaced by two-thirds of the limit of detection (0.001 AU) before log transformation. All

Table 1. Descriptive statistics of selected subject characteristics.

	n (%)/mean (sd)
N	529
Gender (female)	206 (39%)
Smoker*	133 (25%)
Former smoker*	145 (27%)
Age	35.3 (9.6)
Chronic respiratory symptoms	
Asthma	137 (26%)
Bronchitis	46 (9%)
Symptoms of allergy to common allergens	146 (28%)
Symptoms of hyperresponsiveness	78 (15%)
Previous working experience with laboratory animals (yr)	10.4 (9.7)
Yearly hours working with rats (hr/yr) ¶	516 (534)
Cumulative exposure to rat allergen during previous year (ng/m3.hr/yr) GM [GSD] ¶	152 [5.1]
Atopic#	210 (45%)
Sensitized to rats (SPT)#	86 (18%)
Respiratory symptoms of rat-allergy	77 (15%)

* Information on smoking habits was missing for 1 subject.
¶ In 373 subjects reporting to work with rats. Information on exposure to rats was missing for 1 subject.
# SPT = Skin prick test. Results were available for 470 subjects.

statistical analyses were performed using SAS software (version 8.02; SAS Institute, Cary, N.C.).

Exposure response relations for exposure to RUA and levels of IgG4 and for IgG4 levels and sensitization or symptomatic allergy were explored by nonparametric regression modeling (smoothing) using PROC GAM. Atopy and a positive SPT to rat allergens were effect modifiers for the relation between IgG4 and sensitization or symptomatic rat-allergy respectively, and were therefore used to define strata for stratified analyses.

For dichotomous response variables a logistic model was used, and smoothed curves were computed using a logit-link function and transformed to prevalence or cumulative incidence by applying the inverse of the logit-function. The smoothness of the function was initially determined by generalized cross validation (method=GCV), but the number of degrees of freedom was not allowed to exceed a maximum of 3 to avoid extremely irregular curves.

Model selection was done informally by comparing deviances, and favoring the simplest model when there was little difference between two models.

All statistical tests were done 2-sided and a p-value <0.05 was considered significant.

Results

Subject characteristics

Subject characteristics for the study population available in the cross-sectional analysis are presented in table 1. Three hundred and twenty-seven subjects (62%) had been working with laboratory animals for more than 4 years (long-term exposed workers). Prevalences of atopy, rat sensitization, and symptomatic rat allergy were similar for short-term and long-term

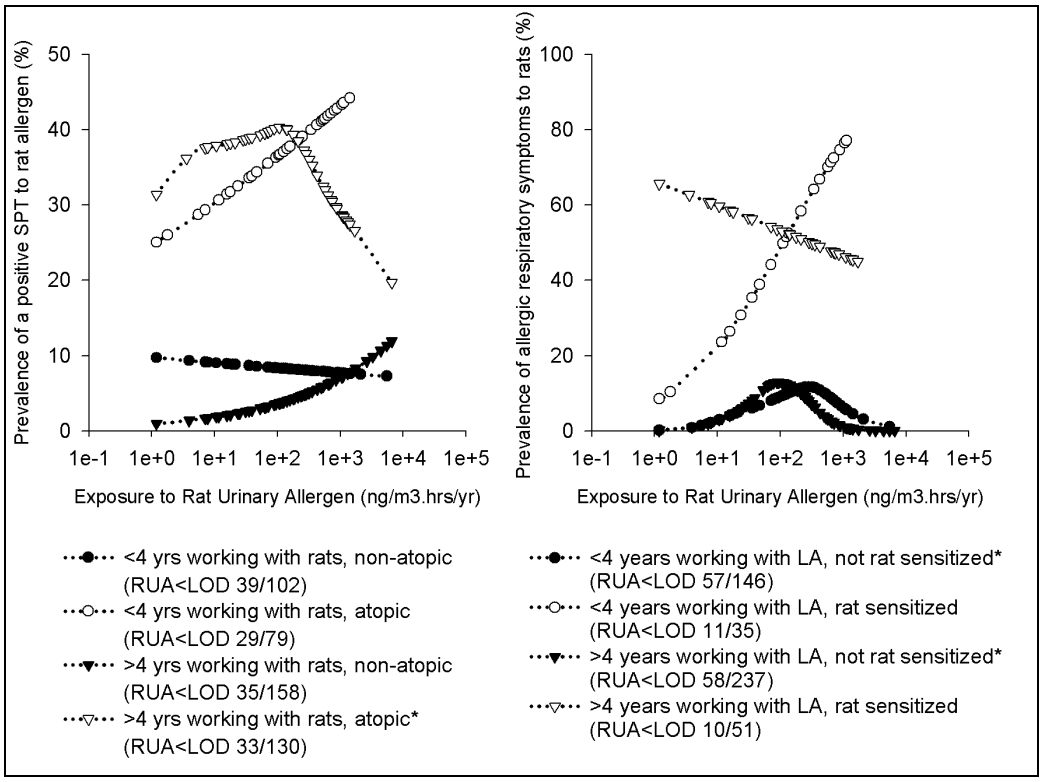

Figure 1. Smoothed (cubic spline) plots of exposure to Rat Urinary Allergen (RUA) and prevalent sensitization (SPT) or symptomatic allergy to rats in long-term and short-term exposed laboratory animal workers. Smoothness of the plot determined by generalized cross validation except where indicated in the legend with a * (see Methods section). Subjects not working with rats during the year preceding the study were assigned an arbitrary exposure of 1.18 ng/m3*hrs/yr before log-transformation (see Methods section). The number of subjects not working with rats is indicated in the legend as: (RUA<LOD number of subjects/total number of subjects in group). For higher exposures a single dot represents from 1-11 subjects (median = 1), depending on the number of subjects with identical estimated exposure in a particular group.

exposed workers, but exposure to RUA was significantly higher in the latter group.

Prevalent rat allergy and exposure to RUA

Using non-parametric models to describe the relation between allergen exposure and sensitization, the strong association between exposure and the prevalence of a positive SPT to rat allergens and symptomatic rat allergy in atopic short-term exposed workers was confirmed (figure 1). For atopic long-term exposed workers risk of sensitization and symptomatic allergy did not increase with exposure to RUA and even declined at the highest exposure levels. Prevalence of a positive SPT and symptomatic allergy were low in non-atopic and non-sensitized workers respectively.

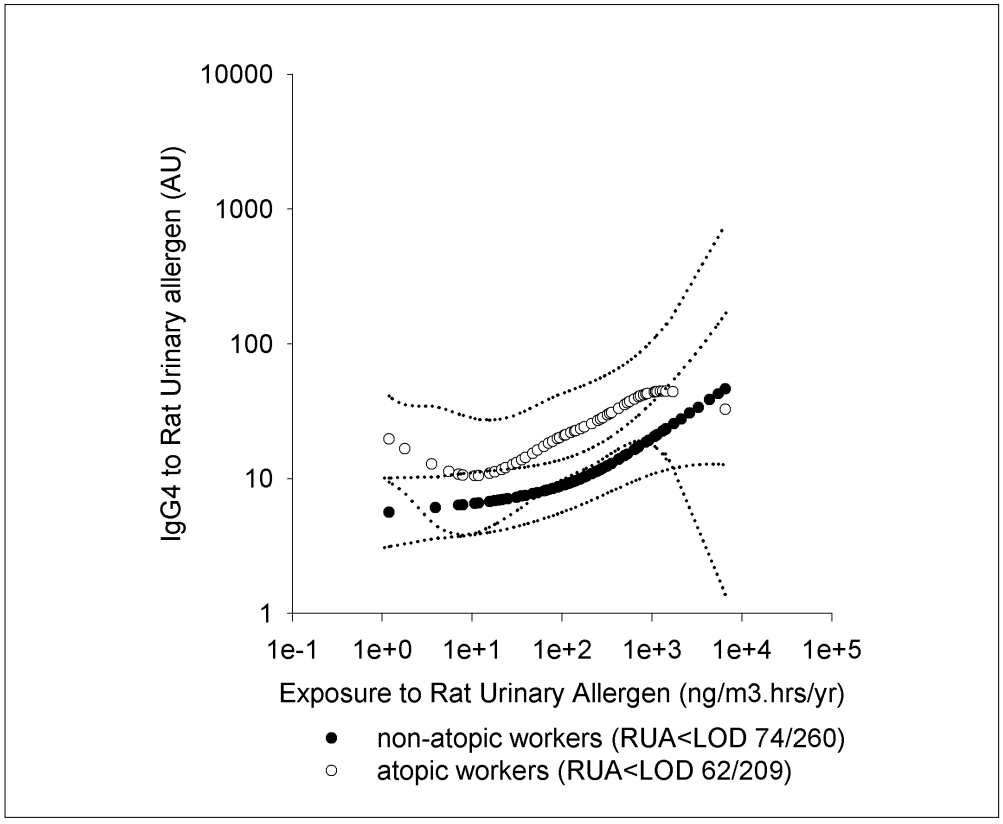

Figure 2. Smoothed (cubic spline) plots with point-wise ±2 SE bands of exposure and IgG4 to RUA in atopic and non-atopic laboratory animal workers. Smoothness of the plot was determined by generalized cross validation. Subjects not working with rats during the year preceding the study were assigned an arbitrary exposure of 1.18 ng/m3*hrs/yr before log-transformation (see Methods section). The number of subjects not working with rats is indicated in the legend as: (RUA<LOD number of subjects/total number of subjects in group). For higher exposures a single dot represents from 1-14 subjects (median = 2), depending on the number of subjects with identical estimated exposure in a particular group.

IgG4 antibody titers and exposure to RUA

IgG4 antibodies to RUA could be detected in sera from 439 of 529 subjects (83%). Log transformed titers followed an approximate normal distribution with a geometric mean and standard deviation (GM [GSD]) of 14.6 [18.4] AU. In nonparametric regression analysis, a clear dose response with exposure to RUA was found for both atopic and non-atopic workers. Atopic workers had higher levels of IgG4 than non-atopic workers, but the shape of the dose response curve was similar for both groups (figure 2). IgG4 levels weakly increased with job duration for subjects who had been working with laboratory animals for less than 10 years, but showed no consistent trend for those with more extensive job histories (not shown).

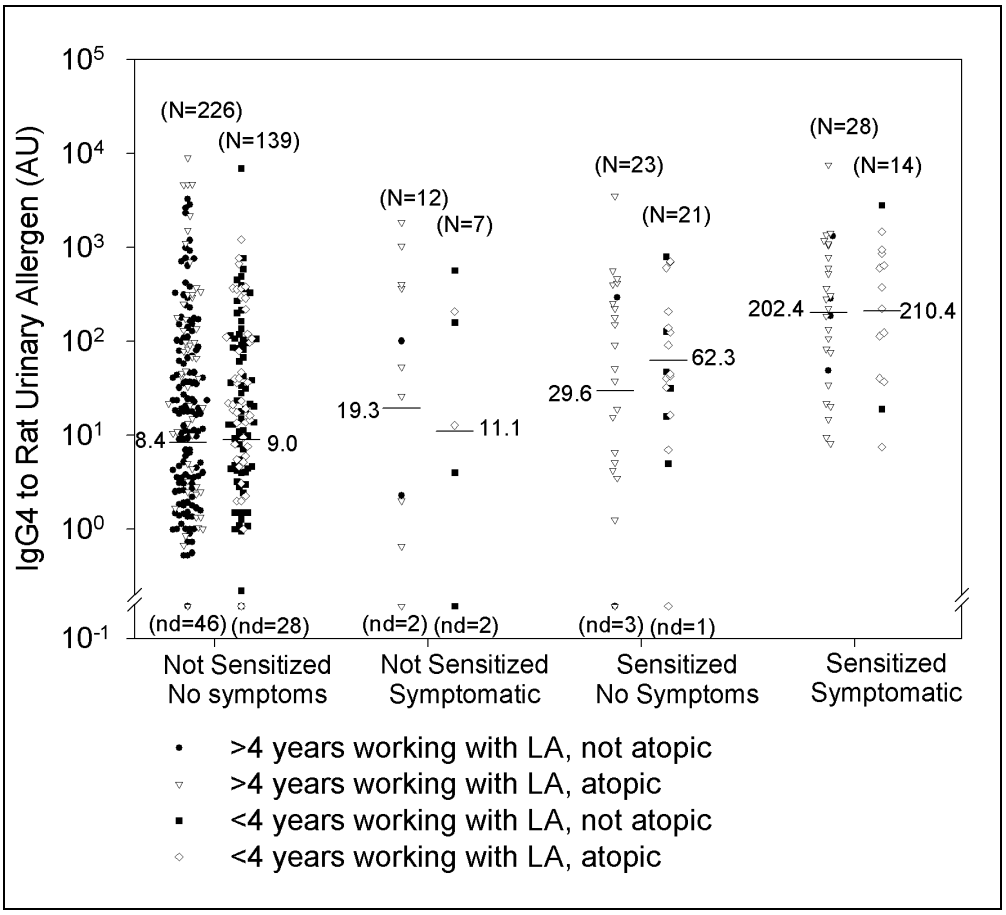

Figure 3. IgG4 to Rat Urinary Allergen (RUA) in sensitized and non-sensitized LA workers with and without respiratory symptoms. N=number of subjects in group. nd=number of subjects with IgG4 below the detection limit.

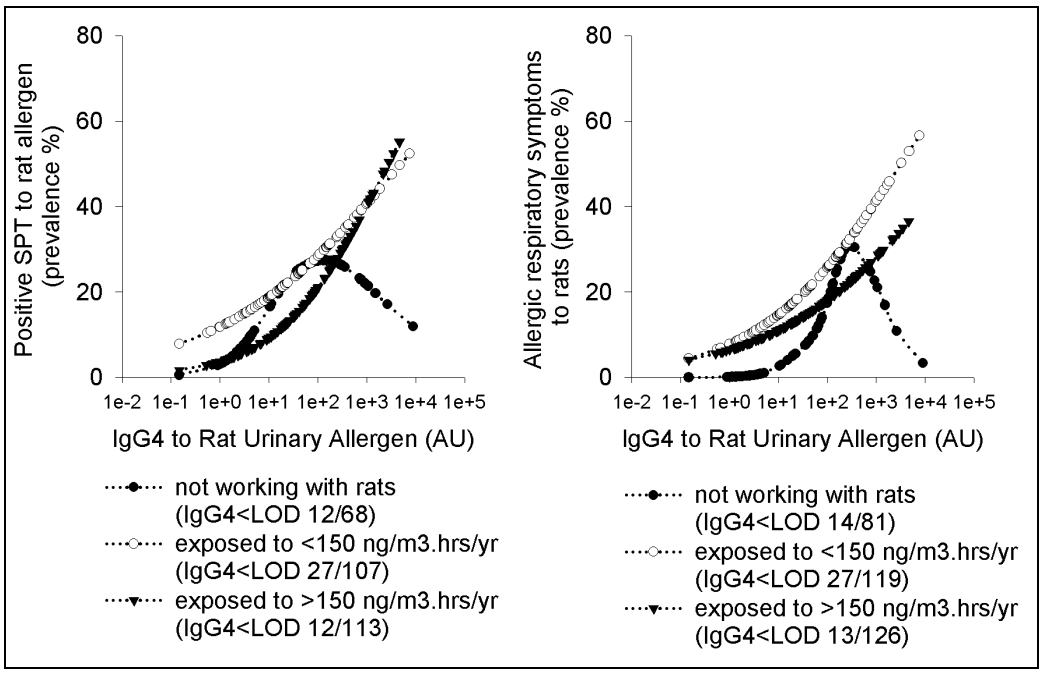

Figure 4. Smoothed dose response curves for IgG4 to Rat Urinary Allergen and prevalent sensitization and symptomatic rat allergy at different levels of exposure to RUA. Smoothness of the plot determined by generalized cross validation except where indicated with a * (see Methods section). Subjects with IgG4 below the limit of detection were given an arbitrary titer of 0.001AU before log-transformation (see Methods section). The number of subjects with IgG4 below the detection limit is indicated in the legend as: (IgG4<LOD number of subjects/total number of subjects in group). For IgG4 titers above the detection limit a single dot represents from 1-4 subjects (90% quantile = 1), depending on the number of subjects with identical IgG4 titer in a particular group.

IgG4 antibody levels and prevalent rat allergy

In a first attempt to assess whether IgG4 was associated with the lack of a dose-response in long-term exposed workers, we compared IgG4 titers in subjects grouped according to the presence or absence of a positive skin prick test (SPT) and/or respiratory symptoms to rat allergens (figure 3). Titers of IgG4 in long-term exposed workers were highest in those that were sensitized and reported respiratory symptoms (GM [GSD] = 202 [5.7] vs. 8.4 [18.3] in non-sensitized, a-symptomatic subjects), but were also slightly higher in sensitized subjects that did not report symptoms (GM [GSD] = 30 [17.9]). Titers were similar for long-term and short-term exposed workers, both within subgroups and overall.

Both weal diameter and serum titer of anti-RUA IgE correlated with IgG4 titers in workers with a positive SPT to rat allergens, although the correlation was not very strong (Spearman ρ=0.34 and 0.32 respectively; P<0.01).

To evaluate whether an inverse association between IgG4 response and rat allergy could exist at very high or very low exposure levels, we further analyzed the association between IgG4 and sensitization or symptomatic rat allergy in long-term exposed workers exposed at three different exposure levels (figure 4). Sensitization risk strongly increased with increasing IgG4 at all exposure levels, and only appeared to level off or decrease at titers above 100 AU in subjects that did not work with rats. The association between IgG4 and prevalence of symptomatic rat allergy within different exposure categories appeared similar, the predicted prevalence of symptomatic allergy leveling off or even decreasing at IgG4 >300 AU in those reporting no contact with rats.

IgG4 antibody levels and incident rat allergy

The association between IgG4 antibody levels and newly occurring sensitization and rat allergy was studied in workers who were not sensitized or did not report allergic respiratory symptoms to rat allergens at the first survey.

Cumulative incidence of a positive skin prick test to rat urine or rat hair in the non-sensitized cohort was 7.9% (25/315) over a period (mean [SD]) of 1.7 [0.5] years. In simple unadjusted analysis both female gender (OR [95%CI] = 3.0 [1.2 - 7.8]) and atopy (OR = 2.3 [0.9 - 5.9]) appeared to be risk factors, while smoking was no relevant explanatory factor (OR = 1.4 [0.5 – 3.5])

An elevated IgG4 antibody level at baseline was a strong predictor of sensitization during follow-up in atopic workers. Sensitization risk increased from 4% (1/27) in those without detectable IgG4 to 28% (9/32) in those with IgG4 > 100 AU (highest tertile of detectable IgG4). In nonparametric regression analysis the association between log-transformed IgG4 and sensitization risk appeared to be well described by a linear logistic model (figure 5). From this model the OR [95%CI] for a 10-fold increase in IgG4 titer was calculated as 2.4 [1.4 - 4.2] for atopic and 1.0 [0.6 - 1.7] for non-atopic workers. These estimates did not change when exposure to RUA was included in the model.

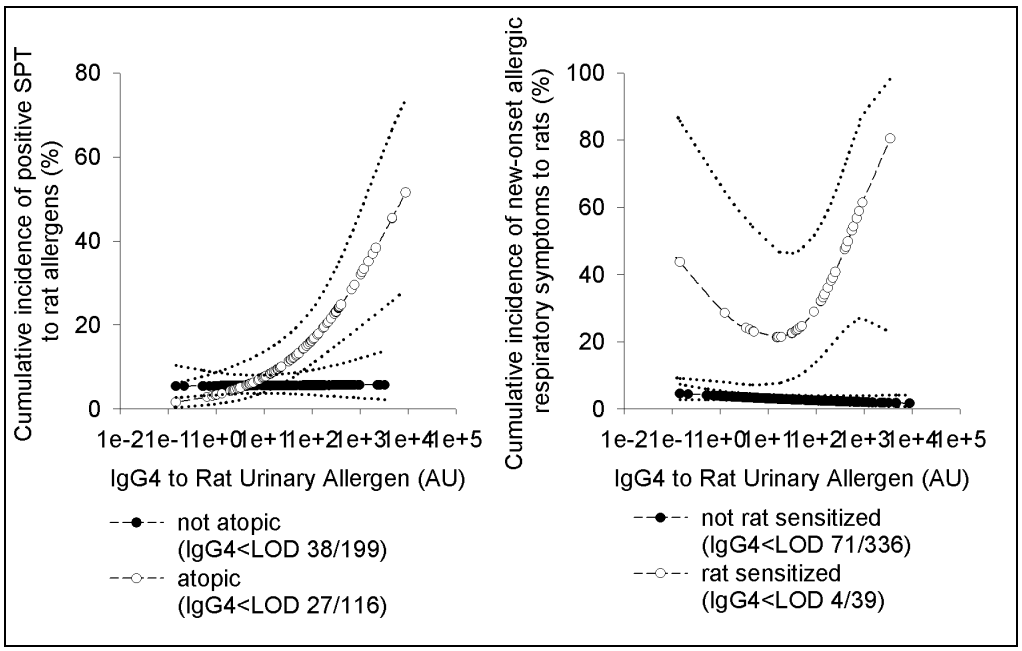

Figure 5. Smoothed dose response curves with point-wise ±2 SE bands for IgG4 to Rat Urinary Allergen and newly occurring sensitization and symptomatic allergy to rats. Smoothness of the plot determined by generalized cross validation. Subjects with IgG4 below the limit of detection were given an arbitrary titer of 0.001AU before log-transformation (see Methods section). The number of subjects with IgG4 below the detection limit is indicated in the legend as: (IgG4<LOD number of subjects/total number of subjects in group). For IgG4 titers above the detection limit a single dot represents from 1-7 subjects (90% quantile = 1), depending on the number of subjects with identical IgG4 titer in a particular group.

Cumulative incidence of symptomatic respiratory rat allergy in the non-symptomatic cohort was 6.8% (27/399) over a period (mean [SD]) of 1.8 [0.4] years. Single most important determinant was a positive skin prick test to rat allergens at the first survey (OR [95%CI] = 16.5 [6.2-44]; SPT results available for 25 cases and 350 non-cases).

The median titer of IgG4 in subjects with detectable levels of IgG4 was 40 AU. Risk of becoming symptomatic in rat-sensitized subjects was high in subjects with titers of IgG4 above this level (10/23 = 43%), lower in those with detectable IgG4 below this level (2/12 = 17%), and highest in subjects with IgG4 below the detection limit (2/4 = 50%). Although not statistically significant and based on just a few observations, this non-linear response was also evident when nonparametric regression analysis was used to model the dose response relation (figure 5) and persisted when subjects with IgG4 below the detection limit were removed from the analysis. Risk of becoming symptomatic was low in those who were not sensitized (approx. 3-4%), and was not associated with IgG4 level.

Discussion

The results of this study strongly suggest that IgG4 antibodies to RUA cannot explain the absence of a dose response between exposure to rat allergens and rat allergy in long-term exposed LA workers. IgG4 antibodies also do not protect against developing respiratory rat allergy. Titers of IgG4 showed a strong and positive dose response with exposure to RUA, and were highest in subjects who were symptomatic and sensitized to rat allergens. Prevalence of a positive SPT or respiratory symptoms to rat allergens did seem to level off or decrease only at the highest IgG4 titers in subjects who did not work with rats. High levels of IgG4 predicted newly occurring sensitization and development of respiratory symptoms in atopic and sensitized workers during follow-up.

This is the first longitudinal study to look at the relation between high levels of allergen-specific IgG4 and development of allergy and allergic respiratory disease in an open working population. This was a relatively large study and exposure to rat allergens had been characterized extensively. However, many subjects had been working with rats for several years prior to enrolment in the study and healthy worker selection may have caused those who were (more) sensitive to the effects of allergen exposure to leave their job either before or during the study. As those lost to follow-up were more often sensitized to rats and also reported respiratory symptoms more often[15], the incidence of sensitization (and symptomatic allergy) as reported here are probably lower than they would have been in a cohort of novice laboratory animal workers.

As subjects were aware of their SPT status at the start of follow-up and symptoms were not confirmed by objective measurements, those with a positive SPT could have been more likely to attribute any respiratory symptoms to working with rats, thus possibly biasing the relation between exposure to rats and onset of respiratory symptoms. It is unlikely that this could explain the association between titers of IgG4 and onset of respiratory symptoms in sensitized workers we found, as this relation was still evident when analyzed in different strata based on (partly self-reported) exposure levels. Use of an arbitrary cut-point for a positive SPT in defining the population at risk for sensitization could have biased our results, since those with a weak SPT reaction who were nonetheless considered SPT negative at the first survey were more likely to become sensitized during follow-up. However, titers of IgG4 were not associated with weal diameter in those with a negative SPT, and exclusion of workers with a marginal skin reaction from the follow-up cohort or using a more stringent definition

for a positive SPT had only minor effects on the strength of the associations we found between IgG4 and rat allergy.

Measurement error may have caused misclassification of exposure and response, but will probably have been non-differential and can therefore not explain why we found positive associations between titers of IgG4 and both prevalent and newly occurring rat allergy.

Although we found a strong association between titers of IgG4 and incidence of sensitization or symptomatic rat allergy, we do not consider IgG4 to be a strong causal factor as these associations were limited to those with a positive SPT to common and/or rat-allergens at the first survey respectively. As reported in other studies[16], presence of IgG4 in subjects that were not sensitized did not increase the risk of becoming symptomatic when working with rats. Several authors have suggested that the presence of high levels of specific IgG4 might be a marker for prolonged and/or high level exposure to the antigen involved[7-9, 17, 18]. The fact that we found a strong association between IgG4 and sensitization or symptomatic allergy even within different RUA exposure strata suggests that there must be additional factors to explain this association. Higher levels of antigen-specific IgG4 in atopic or allergic subjects when compared to non-atopic or non-allergic subjects have also been reported by others[19-22]. As both IgG4 and IgE switching are stimulated by IL4[23] we believe that IgG4 should be regarded as a marker that combines aspects of exposure and susceptibility. This is also suggested by the fact that the dose response between exposure to RUA and titers of IgG4 was similar for non-atopic and atopic subjects, but at different response levels.

Our finding of a strong positive relation between IgG4 and sensitization and symptoms of allergy to rats is at variance with the results for IgG(4) to cat allergen and sensitization and asthma in the study by Platts-Mills and colleagues[5]. In their study IgG(4) to the major cat allergen Fel d 1 was highest in children in the highest exposure category, but was associated with a lower prevalence of IgE sensitization to Fel d 1 and a low risk of asthma. In contrast, IgG(4) to the group 1 mite allergens was positively associated with both exposure and prevalence of sensitization to mite. They suggested that the much higher exposure to cat allergens and lack of significant enzymatic activity of the major cat allergen Fel d 1 might explain these different relations [24]. This hypothesis is not supported by our results as exposure to rat allergens was at least comparable to if not higher than that for cat allergen and the major rat urinary allergens do not appear to possess significant enzymatic activity[25]. The fact that high titers of IgG4 predicted newly occurring positive SPT reactions against rat

allergens also argues against IgG4 being the result of a "*modified*" Th2 response, as strong IgG4 responses in these workers were present before IgE responses became evident.

There are many differences between this study and that by Platts-Mills et al.[5] that could explain our different results. Subjects in our study were older, were probably exposed more intermittently, and exposed to a variety of other chemicals. It is also possible that our use of a crude urine extract instead of purified allergens may have affected our results. It has been suggested that epitope specificity for IgE and IgG4 may be largely different[26] and it is therefore possible that the use of purified allergens would have shown a protective effect of IgG4 antibodies raised against the major allergenic epitopes on prevalent or newly occurring sensitization.

In a recent study by Doekes et al.[27] high levels of IgG4 to Pig Urinary Protein were associated with lower prevalences of respiratory symptoms and BHR in two different populations of pig farmers. However, respiratory symptoms in farmers are often non-allergic and specific sensitization to work-related allergens is rare. Assuming that IgG4 titers are associated with exposure in this population it is difficult to see how a blocking effect of IgG4 antibodies would have a major impact on the presence of primarily non-allergic symptoms. It could therefore be that high levels of IgG4 in this population reflect (relatively) low exposure to agents with a Th1 skewing effect (like endotoxin) that might have more impact on respiratory health for people in this working environment.

Our results are in agreement with the view that an IgG4 response should not be regarded as harmful per se, as high IgG4 titers were not associated with adverse effects in subjects who were not atopic or were not sensitized to rat allergens.

Acknowledgements

Supported by grant no. 99.33 from the Dutch Asthma Foundation.

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Chapter 7

Lung Function Decline in Laboratory Animal Workers: the Role of Sensitization and Exposure

L. Portengen, A. Hollander, G. Doekes, G. de Meer, D. Heederik

ABSTRACT

Objectives Little is known about the relation between allergic sensitization and subsequent long-term lung function changes in working populations exposed to sensitizing agents. We investigated whether exposure and work-related sensitization to laboratory animals were associated with lung function decline.

Methods The relation between exposure and sensitization to laboratory animal allergens and changes in lung function was investigated in a longitudinal study (median follow-up 2.0 years) among 319 laboratory animal workers. Subjects who had been working with laboratory animals for less than 4 years (n=102) were analyzed separately, since an earlier cross-sectional analysis had suggested a strong healthy worker effect in more experienced workers.

Results In multiple regression analyses both sensitization and exposure appeared to contribute independently to lung function decline in subjects who had been working with laboratory animals for less than 4 years, adjusting for gender, age, smoking, and atopy. Lung function decline was most pronounced in sensitized subjects who continued to be in contact with the animals to which they were sensitized, with estimated average excess declines in FEV_1, FVC, and MMEF of 83 mL/yr ($P<0.05$), 148 mL/yr ($P<0.01$), and 7 mL/s/yr ($P=0.9$).

Conclusions We conclude that exposure to laboratory animals is a significant risk factor for accelerated lung function decline, and that sensitized workers are especially at risk.

Occup Environ Med 2003; 60:870-5

Introduction

Clinical studies in sensitized subjects suggest that continued exposure to sensitizing agents can lead to chronic loss in lung function in subjects with occupational asthma [1-3], although this has primarily been evaluated in those exposed to low molecular weight allergens such as toluene diisocyanate [4,5] and Western Red Cedar [6,7].

However, virtually no data are available on long-term changes in lung function from follow-up studies in normal *working* populations and from workers exposed to high molecular weight sensitizers. Flood and coworkers [8] reported similar declines in FEV_1 over 11 years for 198 enzyme-sensitized (i.e. skin prick test positive) and 1484 non-sensitized enzyme detergent workers. Sensitized workers with respiratory hypersensitivity symptoms had been removed from further exposure. Several studies in laboratory animal workers suggest that exposure and/or sensitization to laboratory animal allergens is associated with an accelerated decline in lung function [9-11]. However, most of these studies lacked power and were therefore unable to explore effects of exposure and sensitization separately.

The relation between common atopy, either defined as a positive skin prick test, the presence of serum IgE against common allergens or elevated levels of total IgE, and changes in lung function has been evaluated in several large population [12-18] and occupational [19-21] studies, but with conflicting results.

Weiss et al. [22] investigated the relation between exposure to allergens and longitudinal lung function decline in a selected group of subjects from the Normative Aging Study. Levels of cockroach allergens in house dust at follow-up were significant predictors of annual decline in FEV_1 in both asthmatic and non-asthmatic subjects. Relations between allergen levels and lung function decline were strongest in subjects with a positive skin test reaction compared to those without. No such associations were found for levels of mite or cat allergens.

Altogether these results suggest that lung function may decline more rapidly in subjects who are exposed to allergens, and in particular in those with work-related allergic respiratory disease. We investigated whether exposure and work-related sensitization were associated with lung function decline in a follow-up study among laboratory animal workers. In earlier reports regarding data from the first survey of this study determinants of exposure and the relation between exposure and sensitization to rat and mice have been described [23,24].

Methods

Study design and subjects

Data were used from a cross-sectional survey conducted in 1992/1993, and repeated in 1993/1994 and 1994/1995. Detailed description of the design and methods of data collection have been reported previously [23]. Each survey included a questionnaire, skin prick testing, and lung function measurements. For this analysis only lung function data from the first and last survey were used, as change in lung function over a shorter period may be strongly influenced by short-term variability, leading to loss of power [25].

A complete dataset at the first survey was available for 507 subjects of which 375 (74%) also participated in the last survey. Lung function measurements were available for 319 (85%).

Earlier analyses suggested the presence of a healthy worker effect [23], therefore data from workers who had been working with laboratory animals for more (n=217; 'experienced' workers) or less than 4 years (n=102; 'novice' workers) were analyzed separately. This cut-off point was chosen because most less-experienced laboratory animal workers were Ph.D. students and usually had a contract period of 4 years.

Questionnaire

The self-administered questionnaire contained questions on smoking history, allergic symptoms due to common and occupational allergens, airway hyperreactivity, contact with laboratory animals, and occupational history [26]. For most analyses in this paper only questionnaire data obtained at the first survey were used. Recent exposure to laboratory animals was defined as self-reported contact with laboratory animals, faeces, or urine within the last 12 months. Allergic respiratory symptoms to laboratory animals were defined as the presence of self-reported chest tightness, sneezing, or a runny nose during or after contact with laboratory animals.

Skin prick testing

Skin prick testing was performed as described previously [26] with five common allergens (mixture of *Dermatophagoides pteronyssinus* and *D. farinae*; mixture of grass pollen; mixture of tree pollen; cat fur; dog fur, all from ALK Benelux, Houten, The Netherlands), six occupational allergens (rat urine; rat fur; mouse urine; mouse fur; rabbit fur; guinea pig fur, all from ALK Benelux) and positive (histamine) and negative (PBS) controls. Common atopy was defined as a positive skin prick test (wheal size ≥ 3mm) against at least one common

allergen. Laboratory animal sensitization was defined as a positive skin prick test against at least one occupational allergen.

Spirometry

FVC, FEV_1, and MMEF were recorded with a Vicatest V dry rolling seal spirometer (Jaeger, Breda, The Netherlands). Measurements were performed according to the lung function protocol of the European Community for Steel and Coal [27]. Lung function change was defined as the difference between lung function at the last and first survey divided by the time interval between surveys.

Statistical analysis

All statistical analyses were performed using SAS software (version 6.12; SAS Institute, Cary, N.C.). All continuous variables except the number of pack years followed a normal distribution. Crude differences in prevalence rates or means between groups were compared using Fisher's exact test and Student's *t* test. Differences in number of pack years were tested with a non-parametric rank-sum test (Wilcoxon). Odds Ratios were calculated by logistic regression (PROC GENMOD). The association between lung function change and laboratory animal sensitization, exposure, and allergic symptoms was analyzed by classical multiple regression analysis (PROC GENMOD). Independent variables which were associated (p-value <0.2) both with lung function decline and either exposure, sensitization, or the presence of symptoms to laboratory animals in univariate analysis, were considered possible confounders and included in the regression models, together with known predictors of respiratory function change (gender, age, smoking).

All statistical tests were done 2-sided and a p-value <0.05 was considered significant.

Table I. Subject characteristics.

Parameter	Lost to follow-up (n=132)‡	Available for follow-up (n=375)‡		Actual follow-up (n=319)† Working with animals >4yr (n=217)‡	Working with animals <4yr (n=102)‡	
Gender (female)	51 (39%)	143 (38%)		60 (28%)	56 (55%)	**
Age (yr)	33.6 (9.0)	35.4 (9.8)		39.3 (8.7)	28.6 (7.0)	**
Standing height (cm)	176.8 (8.7)	175.9 (9.1)		176.2 (8.8)	175.9 (10.0)	
Body weight (kilo)	73.9 (12.0)	73.7 (11.9)		74.6 (11.6)	71.8 (12.0)	
Current smoker	44 (33%)	86 (23%)	*	51 (24%)	20 (20%)	
Former smoker	24 (18%)	106 (28%)	*	78 (36%)	15 (15%)	**
Pack years (yr)	4.1 (9.8)	3.9 (7.7)		5.1 (8.7)	1.4 (4.0)	**
Common atopy	61 (46%)	164 (44%)		99 (46%)	45 (44%)	
Chronic respiratory symptoms	48 (36%)	97 (26%)	*	56 (26%)	25 (25%)	
Bronchitis symptoms	19 (14%)	26 (7%)	**	18 (8%)	4 (4%)	
Asthma symptoms	36 (27%)	84 (22%)		47 (22%)	23 (23%)	
History of non-occupational allergy	38 (29%)	96 (26%)		60 (28%)	23 (23%)	
Airway hyperreactivity	22 (17%)	54 (14%)		28 (13%)	17 (17%)	
Years worked with laboratory animals (yr)	8.7 (7.9)	10.8 (10.1)	*	15.9 (9.1)	1.7 (1.1)	**
Recently exposed to laboratory animals	124 (94%)	335 (89%)		203 (94%)	82 (80%)	**
Sensitized to laboratory animals	42 (32%)	102 (27%)		62 (29%)	26 (25%)	
Symptoms of respiratory allergy to laboratory animals	37 (28%)	56 (15%)	**	33 (15%)	15 (15%)	
FEV_1 (%)§	106.9 (11.6)	106.8 (13.4)		108.0 (14.3)	105.9 (11.6)	
FVC (%)§	110.8 (11.8)	111.0 (13.2)		112.8 (14.1)	109.3 (11.7)	*
MMEF (%)§	92.8 (24.1)	91.0 (25.0)		90.2 (25.9)	93.3 (23.6)	

* $P < 0.05$ for comparisons between subjects lost to follow-up and subjects available for follow-up or subjects in follow-up population working with laboratory animals for >4 years and those working for <4 years, respectively

** $P < 0.01$ for comparisons between subjects lost to follow-up and subjects available for follow-up or subjects in follow-up population working with laboratory animals for >4 years and those working for <4 years, respectively

† 56 Subjects who were available for follow-up did not participate in lung function testing

‡ N (%) / Mean (SD)

§ Percentage of predicted value (sex, age-, and height-corrected)[27]

Table II. Univariate regression analysis of annual lung function change over 2 years in Laboratory Animal workers on subject characteristics in: (**a**) subjects working with laboratory animals for less than 4 years, or (**b**) subjects working with laboratory animals for more than 4 years. Continued on next page.

a) Follow-up subjects working <4 years with Laboratory Animals (N=102)

		ΔFEV_1 (mL/yr)		ΔFVC (mL/yr)		ΔMMEF (mL/s/yr)	
Parameter	N	β (SE)		β (SE)		β (SE)	
Gender (female)	56	57.9 (19.4)	**	41.1 (22.8)		85.6 (38.7)	*
Age (/10yr)		-12.5 (14.4)		-9.5 (16.5)		-21.2 (28.3)	
Standing height (/10cm)		-20.9 (9.9)	*	-18.3 (11.4)		-25.2 (19.6)	
Body weight (/10kilo)		-12.1 (8.3)		-14.8 (9.5)		-30.1 (16.2)	
Current smoker	20	-3.4 (25.3)		-13.1 (28.9)		40.4 (49.5)	
Former smoker	15	1.5 (28.4)		-26.5 (32.4)		-5.6 (55.6)	
Pack years (/yr)		1.0 (2.5)		2.3 (2.9)		-0.6 (4.9)	
Common atopy	45	-36.2 (19.9)		-35.5 (22.9)		-81.7 (38.9)	*
Chronic respiratory symptoms	25	-41.4 (23.0)		-4.6 (26.7)		-73.3 (45.2)	
Bronchitis symptoms	4	-80.7 (51.2)		-23.4 (59.2)		-101.2 (101.0)	
Asthma symptoms	23	-31.2 (23.9)		2.6 (27.5)		-66.5 (46.7)	
History of non-occupational allergy	23	-43.2 (23.7)		-27.7 (27.4)		-72.5 (46.6)	
Airway hyperreactivity	17	-31.1 (26.8)		-26.1 (30.8)		-28.9 (52.8)	
Years worked with laboratory animals (/yr)		-12.0 (9.2)		-0.9 (10.6)		-38.1 (17.8)	*
Recently exposed to laboratory animals	82	-48.6 (24.9)		4.9 (29.0)		-76.4 (49.1)	
Sensitized to laboratory animals	26	-48.4 (22.6)	*	-95.4 (24.6)	**	-56.1 (44.9)	
Symptoms of respiratory allergy to laboratory animals	15	-67.0 (27.6)	*	-86.9 (31.3)	**	-24.2 (55.6)	

* = $P < 0.05$
** = $P < 0.01$

Continued on next page….

Results

Subject characteristics

Median time interval between the first and last survey was 2.0 years (range 1.2-2.5). Personal characteristics of all subjects included in the study as well as those who were lost to follow-up are presented in table I. There was no significant difference in the prevalence of sensitization or in gender-, age-, and height-corrected lung function at the first survey, but subjects lost to

Table II. Continued.

b) Follow-up subjects working >4 years with Laboratory Animals (N=217)

Parameter	N	ΔFEV_1 (mL/yr) β (SE)		ΔFVC (mL/yr) β (SE)		ΔMMEF (mL/s/yr) β (SE)
Gender (female)	60	36.5 (14.3)	*	39.6 (17.4)	*	10.1 (35.5)
Age (/10yr)		-14.2 (7.4)		-25.7 (8.9)	**	-4.1 (18.2)
Standing height (/10cm)		-10.7 (7.4)		-9.9 (9.0)		-9.6 (18.1)
Body weight (/10kilo)		-14.8 (5.5)	**	-20.8 (6.6)	**	-7.8 (13.7)
Current smoker	51	-6.5 (15.3)		-5.0 (18.6)		-34.8 (37.4)
Former smoker	78	-4.2 (13.6)		-11.9 (16.4)		6.4 (33.1)
Pack years (/yr)		-2.2 (0.7)	**	-2.1 (0.9)	*	-2.5 (1.8)
Common atopy	99	0.5 (13.1)		1.5 (15.8)		-11.2 (31.9)
Chronic respiratory symptoms	56	7.3 (14.9)		7.8 (18.0)		32.5 (36.2)
Bronchitis symptoms	18	-2.7 (23.6)		31.5 (28.5)		-62.9 (57.4)
Asthma symptoms	47	3.4 (15.8)		11.7 (19.1)		16.4 (38.6)
History of non-occupational allergy	60	15.6 (14.5)		31.5 (17.5)		5.5 (35.5)
Airway hyperreactivity	28	5.3 (19.5)		12.2 (23.6)		20.3 (47.4)
Years worked with laboratory animals (/yr)		-1.3 (0.7)		-1.8 (0.9)	*	-1.5 (1.7)
Recently exposed to laboratory animals	203	-34.0 (26.4)		-24.4 (32.0)		-26.5 (64.6)
Sensitized to laboratory animals	62	-11.2 (14.4)		-6.1 (17.4)		-42.7 (35.1)
Symptoms of respiratory allergy to laboratory animals	33	32.6 (18.0)		61.1 (21.6)	**	0.5 (44.2)

* = $P < 0.05$
** = $P < 0.01$

follow-up more often reported chronic and work-related respiratory symptoms. More female than male subjects had been working with laboratory animals for less than 4 year, and subjects in this group were on average younger, smoked less, and were less frequently exposed to laboratory animals than those who had worked with laboratory animals for a longer period.

Laboratory animal sensitization, recent exposure, and allergic symptoms

A positive skin prick test reaction to at least one of the 4 laboratory animal species was found in 88/319 follow-up subjects (28%). As in earlier analyses [23,24] sensitization to laboratory

animals was strongly associated with common atopy (unadjusted OR [95%CI] = 14 [7-28]), asthma symptoms (unadjusted OR = 3 [2-6]), a history of non-occupational allergy (unadjusted OR = 3 [2–5]), and symptoms of respiratory allergy to laboratory animals (unadjusted OR = 5 [2–10]). Compared to male laboratory workers, female workers were sensitized less often (23% vs. 30%), although this was not statistically significant. Self reported exposure was positively associated with sensitization in subjects working with laboratory animals for less than 4 years for rat allergens only (unadjusted OR [95%CI] = 1.4 [0.4–5.7]). No relation between sensitization and smoking or other subject characteristics at the first survey was found.

Lung function

FEV_1 and FVC at the first survey were somewhat higher than gender-, age-, and height-corrected reference values [27], while MMEF was somewhat lower. No relation between lung function and laboratory animal sensitization or recent exposure to laboratory animals was found, but subjects who reported allergic respiratory symptoms during or shortly after working with laboratory animals tended to have a lower FEV_1 when compared to those without symptoms (mean [SD] = 104% [19%] vs. 108% [12%]; P = 0.07).

Average annual declines in FEV_1, FVC, and MMEF [SD] in the entire cohort (n=319) were -41 [99] mL/yr, -15 [117] mL/yr, and –115 [224] mL/s/yr respectively. In univariate regression analysis lung function decline was significantly associated with gender, age, standing height, body weight, number of pack years smoked, atopy, history of contact with laboratory animals, sensitization against laboratory animal allergens, and symptoms of respiratory allergy to laboratory animals at the first survey (table II). Associations between work-related sensitization or exposure at the first survey and lung function decline were strongest in subjects who had been working with laboratory animals for less than 4 years. Both sensitization and exposure to laboratory animals appeared to contribute independently to lung function decline in multiple regression models, adjusting for possible confounders gender, age, smoking, and common atopy (data not shown). As neither body weight nor standing height was associated with exposure, sensitization, or symptoms to laboratory animals, they were not included in these models. To account for a potential modifying effect of sensitization on the association between exposure and lung function change, a variable indicating whether subjects were sensitized to the animal species they reported working with was added to the model (table III). Lung function had declined most strongly in subjects who were exposed to the animals they were sensitized to.

Table IIIa-b. Multiple regression of annual lung function change over 2 years on laboratory animal sensitization, recent exposure and the presence of respiratory symptoms in (a) subjects working with laboratory animals for less than 4 years, or (b) subjects working with laboratory animals for more than 4 years.

a) Follow-up subjects working <4 years with Laboratory Animals (N=102)

Parameter	N	ΔFEV_1 (mL/yr) β (SE)		ΔFVC (mL/yr) β (SE)		ΔMMEF (mL/s/yr) β (SE)	
Gender (female)	56	44.7 (19.4)	*	14.5 (22.1)		83.7 (40.7)	*
Age (/10yr)		-16.4 (15.3)		-9.8 (17.4)		-22.1 (32.0)	
Pack years (/yr)		0.5 (2.5)		1.9 (2.8)		0.0 (5.2)	
Former smoker	15	30.8 (28.4)		1.3 (32.3)		7.7 (59.5)	
Common atopy	45	-10.2 (20.9)		10.6 (23.9)		-65.3 (43.9)	
Recently exposed to Laboratory Animals	82	-41.1 (25.0)		24.0 (28.5)		-105.9 (52.4)	*
Sensitized to Laboratory Animals	26	11.6 (30.4)		-28.6 (34.6)		-34.7 (63.7)	
Sensitized and exposed to the same Laboratory Animal	13	-91.1 (39.7)	*	-119.4 (45.2)	**	28.4 (83.2)	
Symptoms of respiratory allergy to Laboratory Animals	15	-22.0 (28.6)		-37.7 (32.6)		30.4 (60.0)	

b) Follow-up subjects working >4 years with Laboratory Animals (N=217)

Parameter	N	ΔFEV_1 (mL/yr) β (SE)		ΔFVC (mL/yr) β (SE)		ΔMMEF (mL/s/yr) β (SE)	
Gender (female)	60	25.4 (15.8)		19.6 (19.1)		-2.6 (39.7)	
Age (/10yr)		-6.9 (8.8)		-21.4 (10.5)	*	-3.4 (21.9)	
Pack years (/yr)		-1.5 (0.8)		-0.9 (0.9)		-2.4 (2.0)	
Former smoker	78	10.1 (14.2)		10.1 (17.1)		18.7 (35.6)	
Common atopy	99	-7.3 (15.5)		-17.5 (18.7)		3.3 (38.8)	
Recently exposed to Laboratory Animals	203	-42.7 (26.6)		-39.1 (32.1)		-5.7 (66.7)	
Sensitized to Laboratory Animals	62	-21.5 (25.5)		-22.5 (30.7)		8.2 (63.8)	
Sensitized and exposed to the same Laboratory Animal	44	3.9 (28.0)		8.7 (33.7)		-87.3 (70.1)	
Symptoms of respiratory allergy to Laboratory Animals	33	39.8 (19.5)	*	72.5 (23.4)	*	17.9 (48.7)	

* = $P < 0.05$

** = $P < 0.01$

By adding coefficients for sensitization and for sensitization in combination with exposure, it can be calculated from this table that estimated excess lung function decline in sensitized 'novice' laboratory animal workers who were exposed to the animal species to which they were sensitized was –80 mL/yr (ΔFEV_1), -148 mL/yr (ΔFVC), and –6 mL/s/yr (ΔMMEF), compared to exposed but non-sensitized subjects. A similar analysis involving symptoms of laboratory animal allergy was not possible since only 1 'novice' worker reported symptoms to animals he was not exposed to.

As these associations were based on exposure data collected at the start of the follow-up period, it was considered whether changes in exposure status during the follow-up might have affected these relations. Unfortunately, subgroups that were exposed intermittently were too small to allow meaningful analysis. However, estimated excess lung function decline in 11 'novice' sensitized workers who continually reported exposure to the laboratory animals they were sensitized to was (ß [SD]) -82.6 [36.0] mL/yr ($P<0.05$), -147.7 [37.9] mL/yr ($P<0.01$), and -6.8 [73.0] mL/s/yr ($P=0.9$) for ΔFEV_1, ΔFVC and ΔMMEF respectively, when compared to continually exposed but non-sensitized subjects. Exclusion of never exposed subjects had only minor effects on these estimates, which were very similar to those from the analysis using only exposure data from the first survey.

Discussion

The results of this study suggest that exposure to laboratory animals allergens is a significant risk factor for developing accelerated airflow obstruction, especially in sensitized workers. Estimated average excess declines in FEV_1, FVC, and MMEF were 83 mL/yr, 148 mL/yr, and 7 mL/s/yr in 'novice' laboratory animal workers who were sensitized and were continually exposed to the animal species to which they were sensitized when compared to continually exposed, but non-sensitized subjects. Buist and Vollmer [28] concluded that in order to develop clinically notable airflow obstruction, the average rate of decline in FEV_1 over an adult life would need to be >90 ml/yr or about three times that in non-smokers. This would mean that exposed and sensitized laboratory animal workers in our study are at increased risk for developing clinically relevant airway obstruction. Although there was a clear trend towards increased lung function decline in exposed and sensitized laboratory animal workers, reference groups in some of these analyses were quite small, and the numerical value of the estimates should therefore be interpreted with caution.

Lung function had also declined faster in sensitized 'experienced' laboratory animal workers, but associations with exposure were much weaker and not statistically significant.

Surprisingly, a positive association was found between the presence of symptoms of respiratory allergy and lung function change in workers with more than 4 years experience. However, from these symptomatic workers 15 (45%) did no longer report work-related symptoms at the last survey, despite the fact that most were still working with the animals they had reported symptoms to.

Decline in FVC was paralleled by a similar decline in FEV_1 in most subjects, suggesting that the reduction in FVC was the result of airways obstruction. It has been suggested that MMEF could be a sensitive index to detect mild airflow obstruction [27]. However, changes in MMEF in our study did not correlate well with either changes in FVC or FEV_1. This could be explained by the fact that MMEF is calculated over the middle half of the FVC, and changes in MMEF are therefore hard to interpret when the vital capacity also changes.

The absence of an association between exposure to laboratory animals and lung function decline in sensitized 'experienced' laboratory animal workers may be explained by a healthy worker effect. Subjects who were lost to follow up reported allergic symptoms to common and work-related allergens more frequently and were also more often sensitized to the animals they were working with. When comparing our results with those of other groups it should therefore be considered that we observed the most marked relationships in 'novice' laboratory animal workers.

Misclassification of sensitization, exposure, symptoms, or lung function decline may have occurred in our study but is likely to have been non-differential and will therefore probably have resulted in underestimation of the strength of the associations.

These results confirm and extend some of the findings in the few other longitudinal studies in laboratory animal workers. In a small Swedish study by Renström et al. [9] average changes in FEV_1 and FVC after a median follow up of 18 months were -150 mL and -10 mL in 9 exposed laboratory technician students with laboratory animal allergy (LAA; defined as the presence of IgE and/or symptoms) compared to -50 mL and 0 mL in 29 students without LAA (mean age of subjects at follow-up 25 yr). In the paper by Sjösted et al. [10] decline in lung function was expressed as a percentage of baseline lung function. They reported a decline in FEV_1 of -7.5% over a period of 5 years in 22 exposed workers with laboratory animal-related symptoms and of -2.2% in 27 subjects without chemical or laboratory animal-

related symptoms (mean age of subjects 42 yr). Declines in FVC in both groups over the same period were -2.2% and -1.0% respectively.

Fuortes et al. [11] reported a mean decline in FEV_1 of -328 mL after 24 months of follow-up in a group of 22 previously unexposed animal-workers. This was significantly more than the -132 mL that was recorded in the control group of 16 non-exposed 'wet' laboratory workers. However, loss to follow-up in their study was considerable (78% and 82% in animal exposed and unexposed groups respectively).

Only a modest and not statistically significant association was found between common atopy and lung function decline in this population of laboratory animal workers. Possible explanations include differences in potency, exposure level and temporal exposure pattern between common and work-related allergens. Alternatively, given the strong association between common atopy and laboratory animal sensitization, it seems likely that those atopic workers that did not (yet) get sensitized to laboratory animal allergens are different from those that did and may be less prone to experience allergic reactions or suffer lung function decline.

Based upon their experience with subjects with occupational asthma, Malo and Chan-Yeung [29] have proposed a model in which chronic inflammation develops after sensitization, but possibly before the occurrence of symptoms. From clinical studies investigating inflammatory changes in the lungs of patients with asthma it has become clear that similar changes (but less pronounced) can be found in the lungs of atopic individuals without clinically diagnosed asthma [30-33]. It is therefore possible that lung function decline in sensitized laboratory animal workers is the result of chronic low-level inflammation caused by continued exposure to allergens. Since post-bronchodilator spirometry was not assessed, it is unfortunately not possible to evaluate whether this apparent loss in lung function reflects development of occupational asthma or chronic airflow limitation.

Although small sample size and short follow-up hamper interpretation of these results, exposure to laboratory animals seems to be a serious risk factor for the development of clinically relevant airway obstruction over a period of just a few years, especially in sensitized workers. This may occur in the absence of overt symptoms of respiratory allergy. Because of the implications for occupational hygiene and medicine practice these results will need to be verified in larger and longer follow-up studies.

Acknowledgements:

Supported by grant no. 99.33 from the Dutch Asthma Foundation.

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Chapter 8

General Discussion

Methodological considerations

The studies presented in this thesis all focus on the interaction between host factors and combined exposure to allergens and non-allergenic agents in the development of respiratory allergy in occupational populations. Better understanding of these interactions may provide clues on mechanisms that could be involved in the pathogenesis of respiratory allergy and may help identify groups in which preventive actions can be more effective.[1]

According to Rothman, biological (as opposed to statistical) interaction can be defined as the co-partition in a single causal mechanism of two or more component causes.[1] Any departure from an additive effect on risk or incidence implies presence of biological interaction, although the reverse is not necessarily true. To evaluate the presence of interaction, he recommends entering multiple exposures or host factors into a regression model as a factored set of terms (i.e. a composite variable) and compare estimates of the relative effect for each combination of exposures.[1]

Although this is a useful conceptual model we will not strictly adhere to the analytical details, as allergen exposure measurements were not available for all studies in this thesis. However, several aspects may be relevant to the interpretation of our results. From this definition of interaction as co-partition in a single causal model, there must be interaction between allergen exposure and all other risk factors of allergen-specific sensitization or symptomatic allergy. Since allergen exposure must be a component cause in any mechanism imaginable, there is no risk of sensitization or symptomatic allergy in the absence of exposure. We can therefore assume that biological interaction is present when the risk of sensitization or symptomatic allergy is different for different levels of the risk factor or exposure under study, but at similar allergen exposure levels. Since we did not measure allergen exposure in all studies, we will briefly discuss the likelihood of confounding by differences in allergen exposure when considering results from these studies.

As argued in the introduction (Chapter 1), there is a potential interaction between genes, allergen exposure, non-allergenic exposures, and time. Most of our analyses were performed

on data from cross-sectional studies and no attempt was made to assess familial backgrounds in more detail. We will therefore focus on combined exposure to allergens and non-allergenic agents and only briefly discuss aspects of timing or gene-exposure interactions where appropriate.

To appreciate the potential impact of non-allergenic exposures on the development of respiratory allergy we will first discuss the relation between genetic factors, host factors, or allergen exposure and sensitization or symptomatic allergy to common and work-related allergens, using data from our own studies.

Genetic factors

Genetic factors have been shown to account for as much as 50% of the variation in the prevalence of allergic disease in twin studies.[2, 3] As for any multi-factorial disease, the apparent impact of a genetic factor on asthma occurrence depends on the causal mechanism(s) in which it is involved and the distribution of other risk factors in the population that is studied.[1] Without information on the genetic make-up of their study subjects, investigators often attempt to adjust for potential differences in genetic susceptibility between populations by including a familial or parental history of allergy in multiple regression models. However, when studying the effect of environmental factors on risk of allergy this approach may be flawed, as parents and children share the same environment and may therefore experience the same effects.[4]

In table 1 a breakdown of the prevalence of atopic sensitization to common allergens ('atopy') and self-reported wheeze by parental history of allergy is presented for the different populations that were described in this thesis. Data from the case-control study in pig farmers were not included as the design did not allow valid estimation of these relations in the source population, while female laboratory animal workers were excluded to allow better comparison between the different groups. From this table it is clear that laboratory animal workers reported a parental history of allergy much more often than pig farmers or farming students. It could be argued that this is evidence of genetic differences between the parents of pig farmers and laboratory animal workers, but the difference could also be due to differential reporting or labeling of parental symptoms, or by the fact that parents from subjects in the farmer studies are more likely to live in a rural area.

Table 1. Parental history of allergy and sensitization to common allergens or wheeze. Atopy was defined based on the results from skin prick tests (SPT; Chapter 2, 5-7) or results from IgE serology (Chapter 4) with 5 common allergens. Wheeze was defined as self-reported wheeze ever.

	N	Parental history of allergy Prevalence (%)	Atopy PR [95% CI]	Wheeze PR [95% CI]
Danish farming students (Chapter 2)				
Conscripts	402	20%	2.3 [1.7-3.1]	1.8 [1.1-3.2]
Farming students not born on farm	494	19%	1.4 [1.0-2.1]	1.0 [0.5-1.9]
Farming students born on farm	505	16%	2.3 [1.5-3.7]	3.0 [1.4-6.4]
Cross sectional study in pig farmers (Chapter 4)				
Pig farmers	101	19%	0.3 [0.0-2.3]§	0.8 [0.3-2.0]
§ only 1 of 18 pig farmers with a parental history of allergy was sensitized to common allergens				
Longitudinal study in laboratory animal workers (Chapters 5, 6, and 7)				
'novice' workers	83	34%	0.8 [0.4-1.6]	2.7 [1.2-5.9]
'experienced' workers	223	29%	1.3 [1.0-1.7]	1.4 [0.9-2.3]

Although there was a relation between a parental history of allergy and sensitization to common allergens or wheeze in most of the populations, it did not appear to be very strong. This is in accordance with the conclusion from a recent review of population based studies that evaluated the relation between family history of asthma and asthma in children[5]. Although family history was consistently identified as a risk factor for asthma, the positive predictive value did not exceed 37% and was below 20% in the majority of studies. A parental history of allergy as assessed from self-reported questionnaire data therefore does not seem to be a very good marker of genetic susceptibility, and cannot be used to assess the impact of genetic factors on respiratory allergy in our studies.

In the study in Danish farming students (Chapter 2), non-farming controls and farming students that had not been born on a farm more often reported a parental history of allergy than farming students with a farm childhood (20% vs. 16%; P=0.13). Although not statistically significant, the difference suggests that at least part of the lower risk of atopy and asthma in farmers could be due to pre-job selection. Exposure avoidance by subjects at risk for allergic disease may also have affected results from our study on the relation between disinfectant use and respiratory allergies in laboratory animal workers (Chapter 7). Laboratory

animal workers using disinfectants less often reported a parental history of allergy than those not using disinfectants or using only alcohol (31% vs. 39%; P=0.06).

Host factors

Atopic sensitization to common allergens ('atopy') is a known risk factor for respiratory allergy in the general population[6], while smoking and age are risk factors for prevalence of respiratory symptoms in general.[7] To assess the potential impact of these factors on prevalence of respiratory allergy in our populations we calculated prevalence ratios (PRs) for the relations between smoking and age and atopy, and for the relations between smoking, age, and atopy and wheeze for each of the populations described in this thesis. Results are presented in table 2. As before, data from the case-control study in pig farmers or female laboratory animal workers were excluded.

Atopy was a strong risk factor for wheeze in all populations studied (PRs from 2.0-8.1), while smoking appeared to be an independent risk factor for wheeze mainly in younger workers. Prevalence of atopy tended to be lower in workers over 40 years old, and was strikingly lower in farmers and farming students than in laboratory animal workers. These differences may be partly due to the different methods used to assess atopic status. Prevalence of atopy in laboratory animal workers was lower when assessed by IgE serology (35%) than by skin prick testing (45%), but was still much higher than in adult pig farmers (16%). Atopy was also assessed by skin prick testing in the farming students and rural conscripts, and the differences between these groups and the laboratory animal workers can therefore not be explained by different detection methods alone.

It is unlikely that differences in exposure to common allergens or in genetic background between these populations from different parts of the Netherlands and Denmark could explain the large differences in the prevalence of atopy. The crude prevalence ratio [95%CI] for being a pig farmer instead of a laboratory animal worker and prevalence of IgE-mediated sensitization to common allergens is approximately 0.16/0.35 = 0.5 [0.3-0.7], as estimated from the cross sectional studies in pig farmers and laboratory animal workers. These results clearly show that atopy cannot be considered a risk factor that mainly reflects the genetic predisposition to produce IgE, but that the expression of the atopic phenotype is strongly affected by environmental exposures as well. This will be discussed in more detail further below.

Table 2. Ever smoking and age as risk factors for atopy, and ever smoking, age, and atopy as risk factors for wheeze. Atopy was defined based on the results from skin prick tests (SPT; Chapter 2, 5-7) or results from IgE serology (Chapter 4) with 5 common allergens. Wheeze was defined as self-reported wheeze ever.

	N	Parameter	Prevalence (%)	Atopy PR [95% CI]	Wheeze PR [95% CI]
Danish farming students (Chapter 2)					
Conscripts	402	Ever smoking	34%	1.1 [0.8-1.5]	2.8 [1.7-4.7]
		Age >20	36%	1.1 [0.8-1.5]	1.0 [0.6-1.6]
		Atopy	28%		2.9 [1.8-4.8]
Farming students not	494	Ever smoking	42%	1.0 [0.7-1.4]	3.8 [1.5-5.3]
born on farm		Age >20	25%	1.2 [0.8-1.7]	0.6 [0.3-1.1]
		Atopy	23%		2.2 [1.3-3.5]
Farming students	505	Ever smoking	25%	0.8 [0.5-1.5]	2.8 [1.4-5.4]
born on farm		Age >20	26%	1.0 [0.6-1.6]	0.6 [0.3-1.6]
		Atopy	14%		8.1 [4.1-16]
Cross sectional study in pig farmers (Chapter 4)					
Pig farmers	95	Ever smoking	49%	2.5 [0.9-6.5]	0.9 [0.5-2.0]
		Age >40	63%	0.3 [0.1-0.8]	0.8 [0.4-1.7]
		Atopy	16%		2.5 [1.1-5.6]
Longitudinal study in laboratory animal workers (Chapters 5, 6, and 7)					
'novice' workers	75	Ever smoking	39%	0.8 [0.4-1.4]	1.9 [0.8-4.7]
		Age >40	12%	0.6 [0.2-2.1]	0.5 [0.1-3.1]
		Atopy	39%		2.9 [1.1-7.7]
'experienced'	212	Ever smoking	68%	0.8 [0.6-1.1]	0.8 [0.5-1.3]
workers		Age >40	53%	0.8 [0.6-1.1]	1.7 [1.0-2.9]
		Atopy	47%		2.0 [1.2-3.5]

Allergen exposure

Many studies have shown a clear association between allergen exposure and prevalence of IgE mediated sensitization or symptomatic allergy.[8-17] Occupational respiratory allergy may therefore be considered a useful model to study the role of allergen exposure in the development of non-occupational allergies as well.

Information on allergen exposure was available from the study in laboratory animal workers and detailed analyses of the dose-response curve for time-multiplied RUA exposure and sensitization to rat allergens or rat allergy have been reported earlier.[18] In short, PRs [95%CI]

for exposure to RUA and sensitization to rat allergens for workers with atopy associated risk factors in the high, medium, and low exposure group were 15 [4.0-56], 9.5 [2.1-43], and 7.3 [1.5-37], compared to the prevalence in an internal reference group consisting of workers that were not exposed to rats. Atopy associated risk factors were defined as self-reported allergy, sensitization to cats or dogs, and total IgE > 100 IU/mL. For symptomatic rat allergy, defined as presence of respiratory symptoms attributed to working with rats and sensitization to rat allergens, the association was even stronger with estimated PRs [95%CI] of 42 [5.2-330], 29 [3.2-260], and 7.5 [0.5-120] respectively. These associations clearly illustrate that allergen exposure is a strong determinant of sensitization and symptomatic allergy in susceptible workers.

Another topic that is currently debated in the field of paediatric asthma is whether early life exposure to cats or dogs may protect from development of allergy.[19] One of the hypotheses that has been offered is that high-dose exposure to cat allergen may induce a 'modified' Th2 response, that is characterized by high levels of allergen-specific IgG4 antibodies in the absence of IgE and without symptoms of asthma.[20] In a recent study by Doekes et al. high levels of IgG4 to Pig Urinary Protein (PUP) were associated with lower prevalences of respiratory symptoms and BHR in two different populations of pig farmers.[21] High-dose exposure to rat urinary allergen (RUA) was associated with a strong allergen-specific IgG4 antibody response in our study among laboratory animal workers (Chapter 6). However, anti-RUA IgG4 antibody titers were strongly and positively associated with both prevalent and incident sensitization to RUA and respiratory symptoms attributed to working with rats in atopic and rat sensitized workers respectively. IgG4 to RUA therefore seems to be a marker that combines aspects of exposure and vulnerability in these workers. Not all subjects with high titers of IgG4 were (or became) sensitized to rats, and strong allergen-specific Th2 responses may therefore occur in the absence of a significant IgE antibody response.

These divergent results in pig farmers and laboratory animal workers illustrate the importance of considering etiology when comparing risk factors for respiratory disease between different occupational groups. Prevalence of sensitization to pig allergens in pig farmers is generally low and not associated with presence of respiratory symptoms.[22, 23] Non-allergic reactions to endotoxin and other dust components are therefore thought to be more important for the development of respiratory symptoms in this group.[24, 25] Respiratory symptoms in laboratory animal workers are thought to be mainly due to classical type I allergic responses to rodent allergens.[26] Assuming that IgG4 titers are associated with allergen exposure in pig farmers, it

is difficult to see how this could have a strong impact on the presence of primarily non-allergic symptoms in this population. High levels of IgG4 in pig farmers may reflect low exposure to agents with a Th1 skewing effect like endotoxin, which could explain the apparent protective effect of IgG4 in this population.

These results also raise the question whether monitoring of allergen-specific IgG4 levels in atopic workers should be considered as a means for identifying workers who are at risk to become sensitized to work-related allergens. In our study, cumulative incidence of rat sensitization over 2 years in atopic subjects increased from a low 2% in those with IgG4 antibody titers in the lowest tertile, to 11% in those with intermediate titers and 24% in those with titers in the highest tertile of IgG4.

Most of the studies described in this thesis focus on IgE mediated sensitization to common or work-related allergens as the main health effects. Results from the study presented in Chapter 7 suggest that sensitization is a relevant endpoint, as the estimated excess annual lung function decline in sensitized laboratory animal workers due to working with laboratory animals was larger than that attributed to smoking in general population studies.

Exposure to non-allergenic agents

The importance of combined exposures has long been recognized in risk assessment of chemical agents[27], but little work has been done regarding combined exposure to allergens and non-allergenic agents.[28] Studies investigating the potential health effects of environmental tobacco smoke[29], diesel[30, 31], and endotoxin[32] have recently shown that non-allergenic exposures may have significant impact on the development of respiratory allergy. Combined exposure to allergens and non-allergenic agents is a feature of many work environments, and occupational studies may therefore provide an ideal opportunity to study interactions in more detail, as exposures can be characterized relatively well.

Endotoxin

Many occupations involve exposure to organic dusts containing microorganisms and endotoxin that may have strong pro-inflammatory properties by activating non-specific innate immune responses. Several studies have shown that environmental exposure to endotoxin may significantly modulate expression of atopy[32, 33], but it is generally thought that this occurs mainly or only during the first years of life.[34-36] This has also been suggested by the results

from a large cross-sectional study in children from rural areas in Germany, Austria, or Switzerland. Prevalences of ever having asthma, current asthma symptoms, and atopic sensitization were much reduced when the child had been exposed to stables and farm milk during the first year of life, in comparison to exposure at age 1-5 or no exposure.[37]

We investigated the importance of early life exposures in our study among Danish farming students and controls from the same rural areas (Chapter 2). Farming students and controls with a farm childhood had an approximately 2-fold lower risk of being sensitized to common allergens than those without a farm childhood (PR [95%CI] = 0.6 [0.5-0.8]). This result suggests that the protective effect of a farm childhood may extend well into (young) adulthood. We also found evidence that the effect of a farm environment may not be limited to early life exposure alone, as current attendance of a farming school was also associated with a lower prevalence of atopy independent of a farm childhood (PR [95%CI] = 0.8 [0.6-1.0]). No attempt was made to identify farm-related exposures that might be associated with the reduced risk in this study, but farming students who were in contact with animals (particularly pigs) showed the strongest reductions (unpublished).

It is well known that animal keeping is associated with exposure to high levels of bacterial endotoxin[38], and recent studies have implicated endotoxin exposure as a potential protective factor in the development of atopic diseases in childhood.[33, 39, 40] We used data from a case-control study to analyze the relation between current endotoxin exposure and atopic sensitization in full-time pig farmers (Chapter 3). Even though farmers with symptoms of respiratory disease were over-represented, only 17% were sensitized to one or more of 4 common allergens. A strong inverse relation was found between endotoxin exposure and sensitization to common allergens for exposures up to 75 ng/m^3, with an OR [95%CI] of 0.03 [0.0 – 0.34] for a 2-fold increase in endotoxin. It seems unlikely that that these relations could be explained by differences in allergen exposure or lifestyle factors, as all subjects were full-time pig farmers living in the same rural areas. The most important determinants of modeled exposure were activity patterns involving (close) contact with animals and flooring characteristics, and endotoxin might therefore be a surrogate marker for exposure to other agents of microbial or animal origin in this study.

Together these studies do not support the view that immune responsiveness towards common allergens is already established at an early age and that protective exposures should occur during the initial phase of immune system development.[34-36] The fact that current endotoxin exposure appeared to protect from sensitization to common allergens also

questions the concept that endotoxin exposure should occur before or shortly after first contact with an allergen, as has been suggested on the basis of (limited) evidence from animal experiments.[41] However, the 'protective' effect on atopy in full-time pig farmers was mainly due to a strong reduction in the prevalence of pollen sensitization, and the fact that exposure to these allergens occurs intermittent may be important.

Whether a reduced risk of atopic sensitization also translates into a lower risk of (allergic) respiratory disease is not clear from our results. A farm childhood was associated with a lower prevalence of wheeze, doctor-diagnosed asthma, and bronchial hyper-reactivity (BHR) in the Danish study, but *current* farming and high endotoxin exposure were associated with a higher prevalence of respiratory symptoms and increased BHR in farming students and full-time pig farmers. Endotoxin has strong pro-inflammatory properties, and, from current knowledge, negative effects of endotoxin exposure on respiratory health may well outweigh any potential beneficial effects on allergic sensitization or respiratory allergy.[42]

A further remaining question is whether the protective effect on sensitization is a result of endotoxin exposure or whether this is a proxy for other animal related or microbial exposures.

Disinfectants

Earlier studies in pig farmers as well as general population studies have identified use of disinfectants[43] and cleaning agents[44] as potential risk factors for the development of respiratory allergies. We studied the relation between disinfectant use and IgE mediated sensitization and respiratory outcomes in pig farmers (Chapter 5) and laboratory animal workers (Chapter 6). Our results seem to confirm that disinfectant use may be a risk factor for respiratory allergy in pig farmers. Most of the pig farmers used disinfectants containing quaternary ammonium compounds (QAC's) that were shown to confer the strongest risk in the earlier study.[43] Prevalence of sensitization to common allergens in farmers who did not use disinfectants was very low (6%), and strongly increased with increasing frequency of disinfectant use up to approximately 20% in pig farmers who disinfected at least weekly (PR [95%CI] = 2.5 [0.4-17], adjusted for age and smoking habits). Disinfectant use also appeared to be a risk factor for respiratory symptoms in atopic pig farmers, which also concords with earlier findings.[43] The study suffered from a low participation rate, and selective participation of allergic farmers using disinfectants may have biased our results. The fact that we found no association between a parental history of allergy and atopy or between smoking and wheeze,

suggests that healthy worker selection may have had more impact. This could have been stimulated by government regulations to reduce the size of the pig herd, as this will have provided more (symptomatic) farmers an opportunity to quit farming.

In contrast, results from our study in laboratory animal workers (Chapter 4) do not support a role for exposure to chloramine-T or other disinfectants that do not contain quaternary ammonium compounds (QACs) in the development of allergic disease, at least not at exposure levels customary in laboratory animal work. There was no association between disinfectant use and risk of sensitization or self-reported symptoms to common and work-related allergens in workers who had been working with laboratory animals for at least 4 years prior to the first survey. In workers with a shorter job duration disinfectant use was associated with a reduced risk of sensitization to common and laboratory animal allergens and with a reduced risk of symptomatic allergy. Many of the symptomatic workers not using disinfectants reported their symptoms had started in their early teens, suggesting that selective avoidance of disinfectant exposure by symptomatic workers may have biased these relations. No information was available on the amount or frequency of disinfectant use, but it seems likely that exposure levels were generally much lower than in pig farming, where disinfectant exposure is probably much higher. Differences in exposure to endotoxin and other microbial agents between pig farmers and laboratory animal workers may also be relevant, as an inverse relation between disinfectant use and exposure to microbial agents in pig farmers may explain why we only found an association in pig farmers. However, no relation between disinfectant use and endotoxin exposure was found in the case-control study among pig farmers.

General conclusion

Overall these studies illustrate the importance of combined exposures and interaction effects in the development of occupational respiratory allergy. Allergen exposure is the most important determinant of IgE sensitization or symptomatic allergy in susceptible workers, but non-allergenic exposure to endotoxin or chemical agents seems to be relevant as well. Prevalence ratios for having been brought up on a farm (~0.6), current pig farming (~0.5), and disinfecting more than once a week (~2.5) indicate that non-allergenic exposures may be as important for the expression of atopy as a (self-reported) parental history of allergy (PRs from 1.1-2.3). Exposure to non-allergenic agents may also affect the risk of sensitization to work-related allergens, although this could not be determined in the present studies.

For the prevention of occupational allergy more information is needed regarding the potential of combined exposure to allergens and non-allergenic agents to affect development of occupational allergy. Combined exposures are the rule rather than the exception, and this should be reflected in risk assessment of both allergens and non-allergenic agents. As the associations between endotoxin or other farm-related exposures and IgE mediated sensitization were reported from cross-sectional studies, it is important to investigate whether these relations are causal, and whether a reduced risk of sensitization translates into a lower risk of respiratory disease. This could be done in prospective studies of workers that are newly exposed to high levels of endotoxin and work-related allergens. However, only large, longitudinal studies can provide the information necessary to fully investigate the effects of combined exposures over time.

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Summary

Allergic airway diseases, which include asthma, are of great health concern because of the high morbidity and usual poor reversibility. In epidemiological studies the proportion of adult-onset asthma cases that can be attributed to workplace exposures has been variously calculated to be between 10-25%. Sensitization to work-related allergens and upper airway symptoms are strong predictors for development of asthma, and prevention of work-related sensitization and symptomatic allergy must therefore be the primary goal from a public health point of view. This requires estimation of dose-response relations for allergen exposure and selected health effects and identification of factors that may significantly affect these relations. For most allergens only a proportion of exposed workers become sensitized or symptomatic, even at high exposure levels, which suggests that there are large differences in susceptibility between workers. Atopic sensitization to common allergens ('atopy') is a recognized risk factor for work-related sensitization and occupational asthma due to allergens with a high molecular weight. Atopy has long been considered to reflect mainly genetic factors, but recent studies have shown that the atopic phenotype may be significantly modulated by environmental exposures.

The main objective of this thesis was to study the impact of combined exposure to allergens and non-allergenic agents on the development of respiratory allergy in occupational populations. For most of the analyses data were used that were available from completed studies that investigated risk factors for laboratory animal allergy and development of respiratory symptoms in farming students and pig farmers. One study was designed with the purpose of investigating the impact of disinfectant exposure on respiratory allergy in pig farmers.

In **Chapter 2** we investigated whether a farm childhood and current farming are associated with IgE mediated sensitization and respiratory symptoms in young adults starting a career in farming. Both being a farming student and having had a farm childhood were independently associated with a lower prevalence of sensitization to common allergens. Having lived on a farm during childhood was also inversely associated with self-reported respiratory symptoms and bronchial hyperreactivity (BHR). Direction and strength of the association between being a farming student and respiratory symptoms or BHR varied widely. These results suggest that the 'protective' effect of a farm childhood on atopy and atopic disease can still be found in

young adults, and indicate that current exposure to the farm environment may also be important.

In **Chapter 3** we assessed whether the protective effect of a farm environment can also be found in adult pig farmers and whether it is associated with exposure to endotoxin. Data were from a case-control study in full-time pig farmers, and average long-term exposure to endotoxin was modeled in detail. Only 17% of farmers in this study were sensitized to one or more common allergens, which is almost certainly an overestimate of the population prevalence, as farmers with symptoms of respiratory disease were over-represented. This is almost halve the prevalence reported for males aged 20-70 years in the general Dutch population, or from occupational studies where the same IgE assay was used in bakers, workers in the potato processing industry, and laboratory animal workers. Risk of sensitization strongly declined with increasing exposure to endotoxin from a predicted 70% at the lowest exposure to 20% at an exposure of approximately 75 ng/m3, and remained virtually unchanged for higher exposures.

Disinfectant exposure has earlier been identified as a potential risk factor for atopic sensitization and adverse respiratory health effects in the previous study in pig farmers. Use of disinfectants containing quaternary ammonium compounds (QACs) seemed to confer the highest risk. In **Chapter 4** we investigated the association between disinfectant use and IgE mediated sensitization, markers of airway inflammation, respiratory symptoms, and lung function in a new sample of pig farmers. Despite the presumably frequent exposure to high levels of disinfectants, only one worker showed demonstrable IgE sensitization to QAC's. Prevalence of sensitization to common allergens was only 20%, which is in accordance with other studies that have shown a low prevalence of IgE mediated sensitization in farmers. Disinfectant use was associated with a more than 2-fold higher prevalence of sensitization to common allergens, but this was not statistically significant. Disinfectant use was also associated with respiratory symptoms in atopic farmers, but not with lung function or NO in exhaled breath. Frequent exposure to disinfectants was associated with presence of inflammatory cytokines in nasal lavage (NAL) fluid, but the significance of this is not clear as NALs were obtained at the medical examination, and not directly or shortly after exposure to disinfectants at work. A major drawback of the study was the relatively small sample size, due to a low response rate among farmers. This could largely be ascribed to the outbreak of Classical Swine Fever in the Netherlands in 1997/1998. The low participation rate severely limited the power of the study to detect associations between disinfectant use and health

parameters. It may also have introduced bias due to preferential self-selection of symptomatic farmers using disinfectants into the study, but we found no evidence of significant selection effects.

It is unknown whether disinfectant use may also be a risk factor for atopic sensitization and adverse respiratory health effects in other populations, or whether disinfectant use may be associated with sensitization to work-related allergens. In **Chapter 5** we therefore studied the association between disinfectant use and sensitization to common and laboratory animal (LA) allergens, self-reported respiratory symptoms, and lung function in laboratory animal workers known to be exposed to highly potent rodent allergens. Data were analyzed separately for workers who had been working with LA's for more than 4 years ('experienced' workers) or less than 4 years ('novice' workers). No association was found between the type of disinfectant used and allergic sensitization or respiratory health in 'experienced' workers. Among 'novice' workers prevalences of sensitization to common and LA allergens were both lower in workers using chloramine-T compared to those not using disinfectants or only alcohol. Prevalences of symptoms of respiratory allergy to common allergens and LA's were also lower in workers using disinfectants. However, exposure avoidance by allergic LA workers may have biased these relations as 'novice' laboratory animal workers using disinfectants less often reported a parental history of allergy or presence of allergic respiratory symptoms before the age of 17 than those not using disinfectants. The divergent findings in pig farmers and LA workers may also be explained by different levels of exposure and type of agents used, or by the presumably different etiologies of respiratory symptoms in these groups.

High-dose exposure to animal allergens has been reported to induce immunological tolerance, and may be accompanied by high allergen-specific IgG4 responses. A dose-response relation between exposure to rat urinary allergens (RUA) and allergen-specific sensitization has been shown in laboratory animal workers who had been working with animals for less than 4 years, but not in those with longer job histories. In **Chapter 6** we assessed whether the presence of high levels of IgG4 to rat urinary allergen (RUA) could explain the absence of a dose response relation between allergen exposure and sensitization in these workers and whether high levels of IgG4 could protect from newly occurring sensitization and respiratory symptoms to rats. IgG4 titers to RUA were higher in atopic than in non-atopic subjects, and increased with higher allergen exposure. Titers were highest in workers who were sensitized and reported respiratory symptoms to rats when compared to those who were not. The

strong association between IgG4 and prevalence of sensitization or respiratory symptoms attributed to working with rats appeared to be independent of estimated allergen exposure. IgG4 was a strong predictor of newly occurring sensitization and work-related symptoms to rats during follow-up in atopic and rat-sensitized subjects respectively. Induction of high levels of IgG4 can therefore not explain the absence of a dose response between allergen exposure and allergy in workers with long job histories. However, our results are in agreement with the view that an IgG4 response should not be regarded as harmful per se, as high IgG4 titers were not associated with adverse effects in workers who were not atopic or were not sensitized to rat allergens. We therefore consider anti-RUA IgG4 to be a marker that combines aspects of exposure and susceptibility.

Clinical studies in sensitized subjects suggest that continued exposure to sensitizing agents can lead to chronic loss in lung function in subjects with occupational asthma, but information on long-term changes in lung function from follow-up studies in workers exposed to high molecular weight sensitizers is lacking. In **Chapter 7** we studied the relation between exposure to laboratory animals and longitudinal changes in lung function in sensitized or symptomatic laboratory animal workers. Both sensitization and exposure appeared to contribute independently to annual lung function decline in subjects who had been working with laboratory animals for less than 4 years. In addition, significant and clinically relevant excess lung function decline was observed in sensitized subjects who continued to be in contact with the animals to which they were sensitized. Since post-bronchodilator spirometry was not assessed, it is not possible to tell whether this apparent loss in lung function reflects development of occupational asthma or chronic airflow limitation.

Finally, in **Chapter 8** we discussed the potential impact of combined exposure to allergens and non-allergenic agents on the development of allergy in occupational populations in relation to genetic factors, host factors, and allergen exposure. Although allergen exposure may be the most important determinant of IgE sensitization and symptomatic allergy in susceptible workers, non-allergenic exposure to endotoxin or disinfectants seems to have a strong impact on susceptibility. As combined exposures are the rule rather than the exception in the working environment, these effects are relevant to risk assessment of both allergens and non-allergenic agents.

Samenvatting

Allergische luchtwegaandoeningen, waaronder astma, zijn van belang voor de volksgezondheid wegens de hoge morbiditeit en de geringe kans op herstel. Het aandeel nieuwe gevallen van astma bij volwassenen dat kan worden toegeschreven aan blootstellingen op de werkplek wordt op basis van epidemiologische studies geschat tussen de 10-25%. Sensibilisering voor beroepsallergenen en klachten van de bovenste luchtwegen zijn sterke voorspellers voor de ontwikkeling van astma, en de preventie van beroepsmatige sensibilisering en symptomatische allergie dienen daarom vanuit het oogpunt van de volksgezondheid de hoogste prioriteit te hebben. Dit vereist kennis van de relatie tussen allergeenblootstelling en relevante gezondheidseffecten en informatie over factoren die deze relatie significant kunnen beïnvloeden. Voor de meeste allergenen geldt dat slechts een deel van de blootgestelde werknemers gesensibiliseerd raakt of klachten ontwikkelt, zelfs bij hoge blootstelling. Dit suggereert dat er grote verschillen in gevoeligheid bestaan tussen werknemers. Atopische sensibilisering voor veel voorkomende allergenen ('atopie') is een bekende risicofactor voor beroepsmatige sensibilisering en beroepsastma door allergenen met een hoog molecuulgewicht. Lang is verondersteld dat atopie voornamelijk bepaald werd door genetische factoren, maar recente studies hebben aangetoond dat de expressie van het atopische fenotype ook sterk wordt bepaald door omgevingsinvloeden.

De belangrijkste doelstelling van de studies in dit proefschrift was het bestuderen van het effect van gecombineerde blootstelling aan allergenen en niet-allergene stoffen op het ontstaan van luchtwegallergie in beroepspopulaties. Voor de meeste analyses werd gebruik gemaakt van gegevens die beschikbaar waren van reeds afgeronde studies naar risicofactoren voor proefdierallergie en het ontstaan van luchtwegklachten bij varkenshouders en andere agrariërs. Eén studie werd specifiek opgezet om het effect van blootstelling aan desinfectantia op luchtwegallergie bij varkenshouders te onderzoeken.

In **Hoofdstuk 2** werd onderzocht of het opgroeien op een boerderij of het werken op een boerderij geassocieerd is met IgE sensibilisering en luchtwegklachten in jonge studenten van een agrarische school en een controlegroep van dienstplichtigen afkomstig uit dezelfde streek. Zowel het werken op een boerderij als het opgroeien op een boerderij bleken beide onafhankelijk geassocieerd met een lagere prevalentie van sensibilisering voor veel voorkomende allergenen. Het opgroeien op een boerderij was ook negatief geassocieerd met de aanwezigheid van luchtwegklachten en bronchiale hyperreactiviteit (BHR), maar richting

en sterkte van de associatie tussen het werken op een boerderij en luchtwegklachten of BHR verschilden sterk. Deze resultaten laten zien dat het 'beschermende' effect van het opgroeien op een boerderij op het voorkomen van atopie en atopische aandoeningen nog steeds kan worden aangetoond bij jonge volwassenen, en wijzen erop dat ook meer recente blootstelling aan het boerenbedrijf belangrijk kan zijn.

In **Hoofdstuk 3** werd nagegaan of het beschermende effect van een agrarische omgeving op atopie ook kan worden gevonden bij volwassen varkenshouders en of dit zou kunnen worden verklaard door blootstelling aan endotoxine. Gegevens waren afkomstig van een case-controle studie bij varkenshouders, waarbij de gemiddelde lange-termijn blootstelling aan endotoxine nauwkeurig was gemodelleerd. Slechts 17% van de boeren in deze studie bleek gesensibiliseerd voor één of meer veel voorkomende allergenen, wat vrijwel zeker een overschatting is van het voorkomen in de totale populatie, aangezien varkenshouders met luchtwegklachten oververtegenwoordigd waren. Dit is echter maar de helft van de prevalentie die werd gerapporteerd voor mannen van 20-70 jaar in de algemene Nederlandse bevolking, of bij studies waar dezelfde analysemethode voor IgE werd toegepast in bakkers, werknemers in de aardappelverwerkende industrie en proefdierwerkers. De kans op sensibilisering nam sterk af met een hogere blootstelling aan endotoxine van een voorspelde 70% bij de laagste blootstelling tot 20% bij een blootstelling van ongeveer 75 ng/m3.

In een eerdere studie onder varkenshouders werd blootstelling aan desinfectantia geïdentificeerd als potentiële risicofactor voor atopische sensibilisering en schadelijke effecten op de luchtwegen. Daarbij leek het gebruik van ontsmettingsmiddelen met quaternaire ammoniumverbindingen (QAC's) het meeste risico met zich mee te brengen. In **Hoofdstuk 4** onderzochten we daarom de relatie tussen het gebruik van desinfectantia en het voorkomen van IgE sensibilisering, markers voor de aanwezigheid van ontstekingen in de bovenste luchtwegen, luchtwegklachten, en longfunctie in een nieuwe steekproef onder varkenshouders. Ondanks de vermoedelijk frequente en hoge blootstelling aan ontsmettingsmiddelen bleek slechts één persoon aantoonbaar gesensibiliseerd voor QAC's. De prevalentie van sensibilisering voor veel voorkomende allergenen bedroeg slechts 20%, wat in overeenstemming is met de resultaten van andere studies bij agrariërs. Frequent gebruik van desinfectantia was geassocieerd met een verdubbeling van de prevalentie van sensibilisering, alhoewel deze toename niet statistisch significant was. Het gebruik van desinfectantia was ook geassocieerd met het voorkomen van luchtwegklachten bij atopische boeren, maar niet met longfunctie of het gehalte aan NO in uitademingslucht. Frequente

blootstelling aan ontsmettingsmiddelen was ook geassocieerd met de aanwezigheid van ontstekingsstoffen (cytokines) in vloeistof verzameld door het spoelen van de neusholtes, maar de betekenis daarvan is niet geheel duidelijk aangezien deze spoeling werd uitgevoerd bij het medische onderzoek, en niet direct of kort na blootstelling aan ontsmettingsmiddelen op het werk. Een belangrijke tekortkoming van de studie was de kleine steekproefgrootte door een lage respons bij de varkenshouders. Dit kan voor een belangrijk deel worden toegeschreven aan de uitbarsting van klassieke varkenspest in Nederland in 1997/1998. De lage participatie had een nadelig effect op de statistische kracht van deze studie om relaties tussen het gebruik van desinfectantia en gezondheidseffecten te onderzoeken. Ook kan zelfselectie van symptomatische boeren die ontsmettingsmiddelen gebruikten de resultaten vertekend hebben, alhoewel daar geen direct bewijs voor werd gevonden.

Het is niet bekend of het gebruik van desinfectantia ook een risicofactor is voor atopische sensibilisering en luchtwegklachten in andere beroepsgroepen dan varkenshouders en of het gebruik van desinfectantia ook is geassocieerd met sensibilisering voor werkgerelateerde allergenen. In **Hoofdstuk 5** onderzochten we daarom de relatie tussen het gebruik van desinfectantia en het voorkomen van sensibilisering voor veel voorkomende allergenen en proefdierallergenen, luchtwegklachten en longfunctie in een studie bij proefdierwerkers. Hiervan is bekend dat ze zijn blootgesteld aan zeer potente knaagdierallergenen. Gegevens werden afzonderlijk geanalyseerd voor proefdierwerkers die meer ('ervaren') of minder ('onervaren') dan 4 jaar met proefdieren hadden gewerkt. Er werd geen verband gevonden tussen het soort ontsmettingsmiddel dat gebruikt werd en het voorkomen van sensibilisering of luchtwegklachten bij de 'ervaren' werknemers. Bij 'onervaren' proefdierwerkers kwam sensibilisering voor veel voorkomende allergenen of proefdierallergenen minder vaak voor bij werknemers die chloramine-T gebruikten dan bij degenen die geen ontsmettingsmiddelen gebruikten of alleen alcohol. Ook de prevalenties van luchtwegklachten door blootstelling aan veel voorkomende allergenen of proefdieren waren lager in proefdierwerkers die ontsmettingsmiddelen gebruiken. Mogelijk zijn deze relaties vertekend door het vermijden van blootstelling aan desinfectantia door allergische proefdierwerkers. 'Onervaren' werknemers die ontsmettingsmiddelen gebruikten hadden namelijk minder vaak een familiaire aanleg voor allergie en rapporteerden ook minder vaak allergische luchtwegklachten op een leeftijd dat ze waarschijnlijk nog niet aan ontsmettingsmiddelen waren blootgesteld (jonger dan 17 jaar). Andere verklaringen voor de uiteenlopende bevindingen in varkenshouders en proefdierwerkers zijn de verschillen in blootstellingniveaus, het type gebruikte middelen, of de ontstaanswijze van luchtwegklachten in beide groepen.

Er zijn aanwijzingen dat een hoge blootstelling aan dierlijke allergenen kan leiden tot het ontstaan van immunologische tolerantie, en dat dit vergezeld zou gaan van een hoge allergeenspecifieke IgG4 respons. In een eerdere analyse van de dosis-respons relatie tussen blootstelling aan allergenen in rattenurine (RUA) en specifieke IgE sensibilisering bij proefdierwerkers werd alleen een duidelijk verband tussen blootstelling en sensibilisering gevonden bij werknemers die minder dan 4 jaar met proefdieren hadden gewerkt. In **Hoofdstuk 6** onderzochten wij daarom of de aanwezigheid van hoge titers aan IgG4 tegen RUA het ontbreken van een dosis-respons relatie bij proefdierwerkers met meer werkervaring kan verklaren en of hoge titers aan IgG4 ook geassocieerd zijn met bescherming tegen nieuw optredende sensibilisering en luchtwegklachten door ratten. Gehaltes aan IgG4 tegen RUA waren hoger bij atopische dan bij niet-atopische proefdierwerkers, en namen toe met hogere allergeenblootstelling. Titers waren het hoogst bij werknemers die gesensibiliseerd waren voor ratten en luchtwegklachten rapporteerden na het werken met ratten. Deze sterke relatie tussen IgG4 en het voorkomen van sensibilisering of luchtwegklachten door ratten leek onafhankelijk van de hoogte van de allergeenblootstelling. Hoge titers aan IgG4 waren een sterke voorspeller van nieuw optredende sensibilisering tegen ratten bij atopische werknemers en van het ontwikkelen van werkgerelateerde klachten tegen ratten bij ratgesensibiliseerde werknemers. Hoge titers aan IgG4 tegen RUA kunnen het ontbreken van een dosis-respons relatie tussen allergeenblootstelling en allergie in proefdierwerkers met veel werkervaring daarom niet verklaren. Desondanks hoeft een sterke IgG4 respons niet noodzakelijk als schadelijk te worden beschouwd, daar hoge titers aan IgG4 niet waren geassocieerd met negatieve effecten in werknemers die niet atopisch waren of die niet waren gesensibiliseerd voor ratten. IgG4 tegen RUA zou daarom kunnen worden beschouwd als een marker die aspecten van blootstelling en gevoeligheid combineert.

Klinische studies in gesensibiliseerde patiënten suggereren dat voortdurende blootstelling aan sensibiliserende stoffen kan leiden tot chronisch verlies aan longfunctie bij werknemers met beroepsastma. Er is echter maar weinig informatie uit longitudinale studies over veranderingen in de longfunctie op de wat langere termijn bij werknemers die zijn blootgesteld aan allergenen met een hoog molecuulgewicht. In **Hoofdstuk 7** bestudeerden we daarom de relatie tussen blootstelling aan proefdieren en verandering in longfunctie gebruikmakend van gegevens uit een longitudinale studie onder proefdierwerkers. Zowel sensibilisering als blootstelling aan proefdieren leken onafhankelijk bij te dragen aan de jaarlijkse afname in longfunctie bij werknemers die minder dan 4 jaar met proefdieren hadden gewerkt. Daarnaast werd een significante en klinisch relevante extra daling van de longfunctie

gevonden bij gesensibiliseerde werknemers die in contact bleven met de dieren waarvoor zij gesensibiliseerd waren. Aangezien er geen bronchodilator werd toegediend, is het niet mogelijk te zeggen of dit verlies aan longfunctie te wijten was aan de ontwikkeling van beroepsastma of dat er sprake was van chronische luchtwegobstructie.

Tenslotte werd in **Hoofdstuk 8** het potentiële effect van gecombineerde blootstelling aan allergenen en niet-allergene stoffen op de ontwikkeling van allergie in beroepspopulaties besproken in relatie tot de effecten van genetische factoren, gastheerfactoren, en allergeenblootstelling. Allergeenblootstelling lijkt de belangrijkste determinant van IgE sensibilisering en symptomatische allergie in gevoelige werknemers, maar blootstelling aan niet-allergene stoffen als endotoxine of ontsmettingsmiddelen kan deze gevoeligheid sterk beïnvloeden. Aangezien gecombineerde blootstelling in de werkomgeving eerder regel dan uitzondering is, kan dit gevolgen hebben voor de risicobeoordeling van zowel allergenen als niet-allergene stoffen.

List of publications

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De Waart FG, Portengen L, Doekes G, Verwaal CJ, Kok FJ. Effect of 3 months vitamin E supplementation on indices of the cellular and humoral immune response in elderly subjects. Br J Nutr 1997; 78:761-74.

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Dankwoord

Natuurlijk was ook dit proefschrift er niet gekomen zonder de hulp van begeleiders, vrienden en collega's. In de eerste plaats wil ik daarom mijn promotor Dick Heederik bedanken voor het vertrouwen en de grote mate van vrijheid die ik genoot bij het schrijven van dit proefschrift en de artikelen die eraan ten grondslag liggen. Zijn immer positieve kijk op de zaken heeft mij meer dan eens net dat duwtje gegeven dat nodig was om ook de wat minder aantrekkelijke klussen te klaren. Mijn co-promotor Gert Doekes wil ik bedanken voor de kritische kanttekeningen en kwaliteitsbewaking, maar vooral ook voor het aanbod samen mijn eerste artikel te schrijven.

Many thanks (*tusind tak*) to Torben Sigsgaard and his colleagues from Denmark for allowing me to use data from the SUS study on the respiratory health in young Danish farmers. I always appreciate your sense of humor and good spirit whenever we meet.

Mijn oud-collega's Albert Hollander en Liesbeth Preller ben ik dankbaar voor het vele werk dat zij hebben gestoken in het verzamelen van de omvangrijke datasets waar ik van gebruik heb kunnen maken. Mijn voormalige huisgenoten Jeroen Douwes en Frouwkje de Waard hebben elk afzonderlijk een belangrijke rol gespeeld bij mijn keuze een afstudeervak te doen bij de leerstoelgroep Gezondheidsleer en hebben daardoor mijn interesse in de epidemiologie en de keuze voor een promotie op dit gebied bepaald. Siegfried de Wind, Anoek Besselink en Anjoeka Pronk zou ik willen bedanken voor hun hulp tijdens het veldwerk bij de varkenshouders. Ondanks de soms lange, drukke dagen was de sfeer altijd goed. Anoek heeft geholpen bijna 800 varkenshouders op te bellen om ze over te halen aan het onderzoek mee te doen, terwijl de vele praktische adviezen van Siegfried de kwaliteit van het veldwerk duidelijk ten goede zijn gekomen. Jack Spithoven ben ik erkentelijk voor het diffen van bijna 150 cytospin preparaten afkomstig van de neuslavage. De varkenshouders die hebben meegedaan aan het onderzoek zou ik willen bedanken voor hun tijd en inzet en ik hoop dat de resultaten van het onderzoek bij zullen dragen aan een beter inzicht in het ontstaan van luchtwegproblemen in deze populatie.

Mijn voormalige kamergenote Inge Wouters ben ik dankbaar voor haar hulp en nuttige tips over tal van onderwerpen variërend van neuslavage tot promotie. Alle andere collega's, waaronder talloze kamergenoten, die niet of op een minder directe manier hebben

bijgedragen aan het succesvol afronden van dit proefschrift wil ik in ieder geval bedanken voor hun bijdrage aan de goede sfeer.

Ik ben blij dat mijn broer Renze en Siegfried de Wind bereid waren mij tijdens de promotie als paranimf bij te staan. Tenslotte wil ik mijn vader bedanken voor de hulp bij het ontwerp van de omslag van mijn proefschrift en natuurlijk Marion, Katja en Kim voor het plezier en de broodnodige afleiding die ze mij de afgelopen jaren bezorgden.

Curriculum Vitae

Lützen Portengen werd geboren op 11 mei 1964 te Voorschoten. In 1982 behaalde hij zijn VWO diploma aan het Pieter Groen College in Katwijk. Van 1982 tot 1986 studeerde hij milieuhygiëne aan de Landbouwuniversiteit te Wageningen (LUW), echter zonder deze studie met een diploma af te ronden. Na het vervullen van de vervangende dienstplicht heeft hij in deeltijdstudie de opleiding Natuurwetenschappen richting Voeding en Toxicologie gevolgd aan de Open Universiteit (OU). Na het behalen van dit diploma in 1997 werkte Lützen eerst als projectmedewerker aan verscheidene projecten bij de vakgroep Humane Epidemiologie en Gezondheidsleer van de LUW. In 1999 begon hij aan zijn promotieonderzoek bij de leerstoelgroep Gezondheidsleer aan de LUW en, na overplaatsing van deze groep naar Utrecht, bij het Institute for Risk Assessment Sciences (IRAS). Vanaf 1 september 2004 is Lützen aangesteld als postdoc bij het IRAS waar hij onderzoek zal doen naar de relatie tussen asfaltblootstelling en longkanker bij asfaltwerkers.