Respiratory allergy in agricultural workers: recent developments

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Purpose of review

To review recent evidence on the relationship between occupational exposure to allergens and asthmagens, and risk for developing allergy and asthma and the role of modifying factors in farmers and farm workers.

Recent findings

Recent studies identified agents that have not been associated with allergy or asthma in farming populations before. The most intriguing findings, however, are those that suggest that the development of atopy and asthma might be influenced by exposure to pathogen associated molecular patterns (PAMPs), including endotoxin.

Summary

The adverse and potentially protective effects of microbial exposures in the farm environment are discussed. Recent findings indicate that it is very likely that the protective effect of exposure to PAMPs such as endotoxin is not limited to childhood age. The protective effects that probably developed during childhood can still be observed at adult age. Furthermore, it even seems likely that exposures at adult age (in occupational environments), which stimulate the innate immune system, have an effect on allergy, independently of childhood exposure. It is hypothesized that reversal of atopy might occur as a result of high exposure to endotoxins and other PAMPs.

Keywords

adult age, allergy, atopy, endotoxin, farming, hygiene hypothesis, PAMPs

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Abbreviations

LPS	lipopolysaccharide
OR	odds ratio
PAMP	pathogen associated molecular pattern
$\mathbf{T_{reg}}$	Regulatory T cell
$\mathbf{T_H}$	T helper cell

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Introduction

The classical notion of allergy and asthma in farmers and agricultural workers is that in several sectors of the agricultural industry, workers are exposed to a range of allergens and run a potential risk for developing allergy and asthma. Agriculture is extremely diverse because farmers commonly grow several different crops, and may also raise a range of different animals. Farmers are therefore exposed to a variety of antigens, from plants, animals, insects and moulds, to which they may develop sensitization and become allergic. Recent studies have shown that exposure to chemical agents from farming equipment (diesel fumes), pesticides, welding fumes, etc. may also occur and are associated with elevated asthma risks. The most intriguing observation, however, is that in several large epidemiological studies conducted during the last decade in various regions of the world, the prevalence of atopic sensitization, allergy and asthma is not higher in farmers than in other populations. There is increasing evidence that growing up on a farm may protect against developing asthma and allergic rhinoconjunctivitis, not only during the early years of life, but also at adult age. The evidence for this observation will be reviewed.

Adverse effects of farming on respiratory health

Respiratory health effect in swine-confinement workers is probably one of the best-described adverse health effects in agriculture. Exposure to microbial agents in the swine-confinement environment is associated with an elevated respiratory symptom prevalence, bronchial hyper-responsiveness, accelerated lung function decline and neutrophil-mediated inflammation in the absence of sensitization against swine or food allergens. The evidence for these health effects is mainly based on large series of epidemiological studies conducted in Europe and North America. Recently, Dosman *et al.* [1••] described four female cases of occupational asthma due to exposures in swine-confinement operations. The cases fitted with what is known of the epidemiological studies; they developed symptoms within a few months after first employment and all had markedly increased bronchial hyper-responsiveness. No consistent pattern of skin prick test responses against common or work environment allergens was found. Asthma symptoms and bronchial hyper-responsiveness persisted after cessation of exposure. The inflammatory response was

unfortunately not characterized, but other studies have shown that inflammation develops in non-naive persons exposed to dust from swine-confinement buildings [2,3]. Neutrophilic responses can be observed in workers after an exposure limited to only a few hours [4]. The inflammatory response is orchestrated by alveolar macrophages that carry specific endotoxin-binding receptors, which play an important role in activation of these cells and subsequent inflammatory processes [5]. Interestingly, a study among French dairy farmers indicated that chronic bronchitis was the most prominent health effect, associated with an accelerated decline in lung function over time [6]. Allergy to allergens from the work environment, including cow dander, did not seem to play an important role, contrary to observations from the Nordic countries in Europe, where high allergy and asthma rates are found as a result of cow-dander exposure. Incidentally, clusters of allergy and asthma cases are being picked up for instance among sweet bell pepper workers exposed to pepper pollen [7] and chrysanthemum workers [8]. An important new development on which few firmer data are available is allergies as a result of the increasing use of biological pesticides [9].

Chemical exposures

Studies among smaller and less obvious groups of farmers such as sheep breeders indicate that these farmers might be at high risk for the development of respiratory symptoms, especially symptoms indicative of asthma [10]. Asthma symptoms appeared to be associated with work intensity and chemical exposure during work, e.g. the use of foot baths against foot rot, containing formaldehyde and copper sulfate. The use of foot baths might be a proxy for exposure to endotoxins. The authors concluded, however, that studies including objective measurements should be performed. Pesticides have been shown to play a potential role in the development of wheezing in farmers who participated in the Agricultural Health Study in the USA. The population consisted of a cohort of pesticide applicators in Iowa and North Carolina, enrolled between 1994 and 1997 [11]. The mechanism underlying the symptoms remains unclear and requires further study. In an additional analysis, exposure to diesel and solvents was also associated with an increased wheezing prevalence and these results suggest that these two exposures may contribute also to an elevated asthma risk [12•]. All together, these studies indicate that chemical exposures may play a more important role in the development of asthma, and possibly allergic responses underlying asthma, than believed previously.

The farming environment, atopy and asthma

Numerous studies have shown that atopic sensitization is less probable among farmers' children compared to rural and urban peers. The differences between the populations for (allergic) rhinitis and asthma seem somewhat less convincing. This observation has been established in a series of studies among farmers' children from several countries [13]. An important question is whether the low prevalence is still observable at higher age and whether relationships might be observable in adult farmers. It is commonly assumed that protective exposures with regard to atopy have their effects during the very first years of life. As recently argued, however, there is little evidence to support this [14••]. In the European farmer project, a study among adult farmers, a lower prevalence of allergy was found in adult farmers compared to rural controls [15]. The prevalence of rhinitis and asthma was lower in European (12.7 and 2.8%, respectively) than in Californian farmers (23.9 and 4.7%, respectively), but chronic bronchitis and toxic pneumonitis were more prevalent in Europe (10.7 and 12.2%, respectively) than in California (4.41 and 2.7%, respectively) [16]. These differences in prevalence may reflect qualitative differences in exposure. Exposure in Europe involved risk factors present inside stables (allergens, pathogen associated molecular patterns (PAMPs)), while the US population seemed predominantly exposed to risk factors present in the outdoor farming environment (silica, pesticides).

Intriguing results emerge from a series of very recent epidemiological studies. Portengen *et al.* [17] showed in Danish farmer apprentices and controls that atopic sensitization against a range of allergens was less frequently observed in farmers than in controls. Their evaluation was based on skin prick testing and IgE serology and not on questionnaire data, as in many of the earlier studies among children. Interestingly, current work as a farmer was also associated with a lower prevalence of atopy, independently of childhood exposure history. Although a healthy worker effect can not be ruled out completely, this study strongly suggests that present exposure to agents in the farming environment might be associated with a lower atopic sensitization prevalence.

In the Agricultural Health Study, wheeze associated with animal production, feeds and by-products was evaluated and interactions among susceptible subgroups assessed [18]. Information about the atopic status of the farmers was available from questionnaire data only (self-report of doctor-diagnosed eczema or hay fever). The prevalence of atopy in this study was low (12%), as in other farming populations. The majority of asthma cases were atopic (58%), but a considerable proportion were non-atopic. Logistic regression analysis revealed that wheeze in the past year was associated with raising animals, requiring direct contact (odds ratio (OR) $OR_{dairy} = 1.26$; $OR_{eggs} = 1.70$). A significant dose response was observed for both the number of poultry and the number of livestock on the farm. Farmers who performed veterinary procedures on a

daily basis had an OR of 1.51. Atopy modified the relationship between exposure and wheezing in case of milking cows (OR 5.5, 95% confidence interval (CI) 1.8–11). Also, for farmers with poultry, eggs and beef cattle, a higher odds ratio was observed for wheeze in atopics. Unfortunately, atopy was not considered as an endpoint in separate analyses, so relationships between atopy and contact with animals were not presented in this study.

In a German cross-sectional study among 3112 (response rate 69%) inhabitants aged 18–44 years from a German rural town, a postal questionnaire was used to register information on atopic diseases, life-time exposure to farming environments and potential confounders [19•]. Those with regular childhood exposure to animal buildings had a significantly reduced risk of nasal allergies. The greatest reduction in risk was seen for respondents starting regular visits to animal stables during the first year of life or between ages 3 and 5 years. Participants reporting start of exposure after age 5 remained at a lower risk for nasal allergies (0.70, 95% CI 0.49–1.01) but less strong than the effect observed for those with exposure to the farm environment at a very young age. The authors concluded that the preventive effect of animal buildings' exposure against atopic respiratory diseases continues into adulthood. The magnitude of the effect appears to be age-dependent.

In a Norwegian study among 2169 farmers from different sectors of the agricultural industry, the prevalence of asthma was 3.7% for physician-diagnosed asthma and 2.7% for current asthma (questionnaire-based definition) [20••]. The prevalence of atopy was 14%, but most asthmatic subjects were non-atopic (80%). Compared with farmers without livestock, asthma was significantly more prevalent in cattle farmers (adjusted OR 1.8, 95% CI 1.1–2.8) and pig farmers (adjusted OR 1.6, 95% CI 1.0–2.5). Non-atopic asthma (asthma without sensitization against a series of common allergens) was significantly higher in pig farmers (adjusted OR 2.0, 95% CI 1.2–3.3) and in farmers with two or more types of livestock animals (adjusted OR 1.9, 95% CI 1.1–3.3). The atopic asthma prevalence (asthma accompanied by atopic sensitization against a series of common allergens) was less common in farmers who held two or more types of livestock (adjusted OR 0.32, 95% CI 0.11–0.97). Estimated air exposure levels to endotoxins, fungal spores and ammonia were positively associated with the non-atopic asthma prevalence and negatively with the atopic asthma prevalence. No associations were found between the air exposure levels and atopic sensitization prevalence. The authors concluded that exposure to endotoxins and fungal spores appears to have a protective effect with regard to atopic asthma but may induce non-atopic asthma in farmers. In a comparison with urban and rural populations, the asthma prevalence was lower among farmers (4%) than the two other populations (5.7% in the rural and 7.6% in the urban populations) [21]. The atopy prevalence was remarkably similar in the three populations (9–10%). It appeared that in all three populations, most asthmatics were not atopic (between 67 and 75%, depending on the population). These results were confirmed in a multiple regression analysis. The odds ratio for asthma in farmers was 0.52 (95% CI 0.36–0.75). The odds ratios for atopic asthma (OR 0.33, 95% CI 0.15–0.69) and non-atopic asthma (OR 0.60, 95% CI 0.39–0.93) were also reduced. These results also indicate a protective effect of the farm environment. The observation that the atopy prevalence does not differ between farming and non-farming populations, however, is not in agreement with studies among children. The authors concluded that these findings are indicative of a preventive effect of the farm environment on asthma prevalence, but a healthy worker effect may also play a role.

A case–control study using 100 prevalent farming cases with work-related asthma and rhinitis symptoms from family practices compared with 102 healthy controls was conducted in a rural area in Poland [22]. Controls had to be farmers as well, and did not suffer from any chronic disease. The results suggest that cereal farming and animal breeding were risk factors for respiratory disease in farmers. Cases clearly were more often sensitized against common and work environment allergens than controls. Small non-specialized farms were underrepresented in the cases, possibly because exposures were lower. The authors interpreted the low prevalence of small farmers among the cases as a protective effect. This conclusion can only be made, however, when exposure data become available. Moreover, selection bias, especially of controls, or a differential distribution of cases and controls over family practices might explain these findings and additional analyses are needed before such a conclusion can be drawn.

Finally, among pig farmers in the Netherlands, it was shown that current endotoxin levels, measured by personal sampling, were associated with a lower prevalence of atopic sensitization [23••]. The effect was especially present at endotoxin levels above 750 EU/m^3 during the working day. Such exposures are, compared to other occupational environments, considered as relatively high exposures. It is unlikely that this observation is explained by being raised on the farm, because most pig farmers have been raised on the farm, so these results are more suggestive of the potentially protective effect of endotoxin exposure on atopy. Interestingly, elevated endotoxin levels were associated with a reduced lung function, increased bronchial hyper-responsiveness and an elevated symptom prevalence.

Causal factors

A major question is which agents might explain the negative association between atopy and the farming

environment exposures. Eduard *et al.* [20] found associations with exposures to bacterial endotoxin, mould exposure and ammonia, especially the associations found with bacterial endotoxin and mould exposure fit in the concept of what is considered the hygiene hypothesis [13]. It seems most likely that ammonia does not play an etiologic role but exposure levels correlate with other agents present in the indoor environment. The farming environment is a complex environment in which a wide range of microorganisms and PAMPs may be present in high levels. Pro-inflammatory agents from Gram-positive bacteria or moulds, such as peptidoglycans or $\beta(1-6)$glucans, may be equally important as endotoxins. This has recently been underpinned in a study of TOLL-like 2 receptor polymorphism and atopy in German children. In this study, it was shown that only farmers' children with at least one wild-type allele were protected against allergy. Farmers' children homozygotic for the mutant (inactive) allele had the same prevalence of atopy as the urban population [24]. Also, bacterial DNA motifs containing CpG oligodeoxynucleotides may modify the atopic response. Bacterial DNA levels were measured in several environments and highest levels were measured in farm barns (mean, 22.1 μg/g dust), followed by rural homes (6.3 μg/g), farm homes (2.2 μg/g; 0.1–9.1) and urban homes (0.6 μg/g; 0.1–1.2). Farm-barn DNA significantly potentiated lipopolysaccharide (LPS)-induced responses in an assay using peripheral blood mononuclear cells. DNA from six urban homes did not demonstrate this effect [25••]. Radon *et al.* [26•] considered infectious agents as candidates for protective effects (*Toxoplasma gondii* or *Helicobacter pylori*) but did not find associations with atopy.

Possible mechanisms

There are two possible ways in which exposure to microorganisms can induce a lower atopy prevalence: (i) by increasing the shift from the $T_H2 \rightarrow T_H1$ phenotype, or (ii) by suppressing the T_H2 response. In a recent survey, it is hypothesized that both the diminished IL-12 and IFNγ production leading to a reduced T_H1/T_H2 ratio and the reduction in regulatory T-cell (T_{reg}) activities will favor a T_H2 response. As a consequence, a lower microbiological burden will result in a higher probability for development of a T_H2 than of a T_H1 response, explaining the relative increase in allergies compared to the autoimmune diseases in the westernized communities [27••].

$T_H2 \rightarrow T_H1$ shift

Bacterial endotoxins are strong immuno-modulating substances that can inhibit an atopic T_H2 response by promoting and enhancing T_H1 responses through production of T_H1-polarizing cytokines IL-12 and IFN-γ from cells involved in the innate immunity [28]. These protective effects for endotoxin may be modified by variations in the gene-encoding TLR4 – an important LPS receptor on antigen-presenting cells, macrophages and monocytes [29]. The first polymorphism related to LPS susceptibility was demonstrated in the study by Schwartz *et al.*, who found that a few co-segregating missense mutations in the *TLR4* gene were responsible for LPS hypo-responsiveness in humans. The allele frequency was around 8% in a rural Iowa population [30]. Polymorphisms for another component of the LPS–LPS binding protein complex, CD14, have also been studied as a risk factor for asthma; however, the results have been conflicting [31,32].

Suppression of the T_H2 response

Suppression of the immune response by cell-to-cell contact via the $CD4^+$ $CD25^+$ T_{reg} cells (mainly involved in regulation of autoimmunity) or by the release of IL-10 and TGF_β by T_{reg} of the adaptive immune system is another important way of regulating the immune response [33••]. The evidence for a down-regulation of the immune T_H2 response by T_{reg} lymphocytes is scarce. The fact that the in-vitro response to LPS in children from farmers was attenuated could be an effect of regulation [34]. It also might be an effect, however, of up-regulation of other PAMP-related mechanisms as bactericidal/permeability-increasing protein [35] and palate, lung and nasal epithelial clone (PLUNC) [36] in these chronically exposed children, as known from another study on the same group of farming children [37]. One study points towards down-regulating of an established immune response [38], demonstrating a significantly higher loss of skin-prick reactions among children from farms compared to non-farm children. This study only included, however, 11 farm children with a positive SPT reaction at the start of the 3-year follow-up period. Finally, a recent study has shown that the effect of IL-10 on the T_H2 system is on the direction of the response, rather than suppression. Pollen-induced IL-10 increase was shown to divert the effect away from IgE to IgG_4 [39•].

Conclusions

Although findings are not always consistent, several studies suggest that in addition to endotoxin, other microbial factors may be associated with a low atopy prevalence. Further research focused on identifying potentially relevant agents in the farming environment is needed. There is also some epidemiological evidence for potential protective effects of microbial exposures, especially endotoxin, with regard to atopy and atopic asthma at adult age. More information is needed – especially studies that include quantification of microbial and allergen exposures and atopy measured by serological tests or skin-prick tests. These results are in agreement with a limited number of animal studies that have been conducted [40,41•]. The studies which indicate that endotoxin exposure may protect against atopy and (atopic) asthma

were conducted in adult mice. Epidemiological studies also suggest that asthma, especially non-atopic asthma, seems to increase with increasing exposure to microbial agents in the agricultural work environment. These indications require follow-up and new studies that focus on a better characterization of the asthma phenotype modification by genetic factors such as polymorphisms related to Toll-like receptors. Especially, longitudinal studies among workers entering the industry are needed to be able to evaluate whether reversal of atopy is a likely explanation of the findings that are now available from cross-sectional studies. Although small, one study in children suggested that high exposure to endotoxin might lead to reversal of the allergic status as well as a lower incidence of new sensitization. Especially, studies among (young) adults, entering and leaving work environments with high endotoxin exposure, are appropriate to evaluate this further.

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