ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2001, 8, 255–259

YOUNG FARMERS WITH CELLULAR REACTIVITY TO AIRBORNE MICROBES SUFFER MORE FREQUENTLY FROM WORK-RELATED SKIN SYMPTOMS AND ALLERGIC DERMATITIS

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Śpiewak R, Skórska C, Góra A, Horoch A, Dutkiewicz J: Young farmers with cellular reactivity to airborne microbes suffer more frequently from work-related skin symptoms and allergic dermatitis. *Ann Agric Environ Med* 2001, **8**, 255–259.

Abstract: 75 farming students (49 males and 26 females aged 16–23 years) underwent dermatological, laryngological and pulmonary examination, skin prick tests with common and farm allergens, Phadiatop and total IgE measurement. After that, the migration inhibition tests with antigens of airborne microbes typical for farm environment (*Saccharopolyspora rectivirgula*, *Pantoea agglomerans*, and *Aspergillus fumigatus*) were carried out. Possible differences between students with positive results and those non-reactive were sought. Results: 10 students reacted to at least one microbial antigen in the migration inhibition test. There were no significant differences in distribution of atopy, prick test results, total IgE, and Phadiatop between the reactive students and their classmates. Only one case of asthma was found, hence a further statistical analysis was not feasible. Allergic rhinitis has been found in 30% of the reactive and in 9.2% of non-reactive students; the difference, however, was not statistically significant ($p = 0.06$). Significant differences were found with respect to the frequency of allergic skin diseases (40% reactive *versus* 9.2% non-reactive, $p = 0.009$); no other triggering factors than microbial antigens could be identified in 2 out of 4 reactive students with dermatitis. Work-related symptoms were present in all reactive students (100% *versus* 27.7%, $p = 0.001$); 8 out of 10 reactive students did not show any other specific sensitisation. Antigens of airborne microbes are commonly associated with lung diseases. Our results, however, suggest that the skin may be affected as well. Relatively strong association between cellular reactivity to airborne microbes and skin diseases deserves further studies.

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Key words: young farmers, work-related symptoms, allergy, skin disease, working environment, airborne bacteria and fungi, microbial antigens, cellular reactivity, migration inhibition test, Lublin Study.

INTRODUCTION

Airborne bacteria and fungi present in the farmers' working environment have been widely recognised as important agents causing occupational lung diseases (reviewed in [12]). In a recent study, Prior and colleagues have suggested that sensitisation to the microbial antigens may take place already in the first years after starting work as a farmer [21]. The aim of our study was to assess the prevalence of sensitisation to microbial antigens on the cellular level among students of agricultural schools in eastern Poland. Additionally, the relevance of this sensitisation was assessed by comparing the health status of students sensitised to microbial antigens *versus* their non-reactive classmates.

Received: 29 October 2001
Accepted: 3 December 2001

MATERIALS AND METHODS

Study group. 75 students of five agricultural schools in eastern Poland were included in the study, 49 males and 26 females aged 16–23 (median 19) years. In each school, in classes with the profile "agricultural production" the first 15 persons from the lists of students who had lived on a farm were asked to participate in the study. All participants were informed about the procedures and possible risks and gave a written consent for taking part in the study.

Medical examination. A dermatologist, a laryngologist, and an internist carried out the medical examination, focused on allergic diseases of the skin and upper and lower airways. Special attention was given to any work-related symptoms from these systems. In addition, rhinoscopy and spirometry were carried out. Skin prick tests, both with ubiquitous and farm-typical allergens (mites *Dermatophagoides pteronyssinus*, *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, grain dust, straw dust, hay dust, cow epithelium, pig epithelium, horse epithelium, grass pollen, animal dander, tree pollens, weed pollens) were also carried out. Subsequently, two blood samples were collected from each student. In one of these samples, determination of total serum IgE and Phadiatop test was carried out using Pharmacia UniCAP 100® apparatus. The students were classified as atopic if at least one of the following criteria was fulfilled: 1) positive Phadiatop test, 2) total IgE above 120 kU/l and at least one positive reaction in skin prick tests, or 3) three or more positive reactions in skin prick tests. The second blood sample was taken by EDTA-precoated syringes for the migration inhibition tests with microbial antigens.

Preparation of microbial antigens for testing. Migration inhibition test were carried out with antigens of three microbes, which are present in high concentrations in the farming environment: thermophilic actinomycete *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni, Faenia rectivirgula*), Gram-negative bacterium *Pantoea agglomerans* (syn. *Erwinia herbicola, Enterobacter agglomerans*) and filamentous fungus *Aspergillus fumigatus.* The antigens for testing were prepared according to the procedure described earlier [24]. Bacterial masses of *S. rectivirgula* and *P. agglomerans* were harvested from nutrient agar cultures, while *A. fumigatus* was harvested from sugar broth culture. The microbial mass was then homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hours, concentrated by evaporation to 0.1–0.15 of previous volume and lyophilised.

Test for inhibition of leukocyte migration in the presence of specific antigen. The whole blood migration inhibition test according to the method of Bowszyc *et al.* [6] was carried out with the antigens of *S. rectivirgula*, *P. agglomerans* and *A. fumigatus*. 0.5 ml of patient's blood and 0.12 ml Parker's culture medium were each added to two silicon test tubes. After that, 0.12 ml of the microbial antigen solution in the concentration of 100 μg/ml was added to one tube, while 0.12 ml of the PBS was added to the other one (as negative control). Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised capillary test tubes 75 × 1 mm. The tubes were sealed at both ends with a 4:1 mixture of paraffin and Vaseline, centrifuged for 10 min at 1500 rev/min and fastened on microscopic slides with adhesive tape at an angle of 10°. The microcultures were then incubated for 4 hrs at 37°C in a humid chamber. The leukocyte migration distances, visible as distinct white zones, were measured under a binocular microscope. Each test was repeated in at least 10 tubes. The results were expressed as a migration index (MI), i.e. the ratio of the mean migration distance of leukocytes in microcultures with antigen, to the distance in microcultures without antigen. The test was considered as positive if the MI was equal to or lower than 0.79 [18].

Statistical analysis. Depending on the results of the migration inhibition test, the students were divided into two subgroups. The possible differences between both groups regarding frequency of atopy, allergic diseases and work-related symptoms were analysed using the chi-square test.

RESULTS

Ten of the 75 farming students (13.3%) had positive migration inhibition test results with at least one microbial antigen. Seven students reacted to *S. rectivirgula*, three – to *P. agglomerans*, and two – to *A. fumigatus* (Tab. 1). Comparison of reactive *versus* non-reactive students is presented in Table 2.

The occurrence of positive cellular reactions to environmental microbial antigens in the examined farming students was significantly correlated with work-related symptoms ($p = 0.001$) and skin diseases ($p = 0.009$). No significant relationship was found between occurrence of these reactions and atopy or IgE-mediated reactions to ubiquitous allergens.

DISCUSSION

During the last 30 years, intensive research has been conducted on dust-related pulmonary diseases in farmers, which resulted in good knowledge on the pathomechanisms and classification of causative substances and microbes [12, 17]. Bacteria and moulds, as well as their biochemical components, excretions, toxins and antigens, are substantial components of organic dust on farms [1]. Recently, it has been shown that precipitating antibodies to microbial antigens may be found already in students of

Table 1. Clinical and laboratory findings in students reactive to microbial antigens in the test for inhibition of leukocyte migration (ILM).

Student No	Gender	*S. rectivirgula* ILM	*P. agglomerans* ILM	*A. fumigatus* ILM	Prick tests	Phadiatop test	Total IgE	Atopy	Skin disease	Nose disease	Work-related symptoms
1	F	+	–	–	–	–	↑	–	–	–	Pruritus of uncovered skin provoked by grain dust
2	F	–	+	–	–	–	N	–	ACD	SAR	Itchy rash of hands and forearms while picking strawberries
3	M	+	–	–	–	+	↑	+	–	–	Nasal blockage and cough provoked by grain dust and hay dust
4	F	–	+	–	*A. siro*	+	N	+	ACD	–	Pruritus, papular skin rash and rhinitis while working in cow barns
5	F	+	–	–	–	–	N	–	–	PAR	Pruritus and erythema of forearms provoked by hay dust
6	M	+	–	+	–	–	N	–	AD	–	Pruritus and erythema of uncovered skin while exposed to grain dust
7	M	–	–	+	–	–	N	–	–	–	Pruritus and papular rash after work in horse stables; itchy nose and rhinorrhea during haymaking and unloading dry hay
8	M	+	–	–	*Derm. pter.*	+	↑	+	–	PAR	Sneezing and cough during unloading dry hay or grain
9	M	+	+	–	–	+	N	+	AD	–	Pruritus and erythema provoked by grain dust
10	M	+	–	–	–	–	N	–	–	–	Pruritus, erythema and rhinitis provoked by grain dust

Derm. pter. – *Dermatophagoides pteronyssinus*, N – result within normal range (≤ 120 kU/l), ↑ – result above normal range (> 120 kU/l), AD – atopic dermatitis, ACD – allergic contact dermatitis, SAR – seasonal allergic rhinitis, PAR – perennial allergic rhinitis.

farming schools [21]. This was confirmed by our study: among 75 random students of agricultural schools, 10 (13.3%) showed positive cellular reactions to microbial antigens.

Correlation between cellular sensitivity to airborne microbes and allergic diseases. We were not able to find a correlation between the specific reactivity and pulmonary symptoms in the tested population. In the whole group, only one case of asthma was found (in a non-reactive student). The number of observed cases was therefore not sufficient to draw any statistically valid conclusions. There are several possible explanations for this. Firstly, the presence of cellular reactivity may be present before any symptoms become apparent, and in some instances the symptoms may never appear. Secondly, the presence of cellular reactivity usually does not imply the type of clinical response (IgE-, IgG-mediated, others). With respect to the nasal symptoms, allergic rhinitis was more prevalent in the group of reactive students (30% *versus* 9.2% non-reactive classmates); the difference, however, failed to prove statistically significant ($p = 0.06$).

Significant differences have been found with respect to the frequency of allergic dermatitis, which has been found in 4 out of 10 (40%) students with positive cellular reactions to microbial antigens, compared to 6 out of 65 (9.2%) non-reactive classmates ($p = 0.01$). We found four farming students reactive to microbial antigens who had had dermatitis (Table 1: Nos 2, 4, 6, and 9). In two cases, possible allergens were traced (in student No 2 a history of nickel allergy; in student No 4, positive prick test to *Acarus siro*). However, in the two remaining students (Nos 6 and 9), the only traceable specific reaction was the cellular reactivity to environmental microbial antigens. In our previous study, 14 hop farmers complained of work-related skin symptoms. Three of them reacted on prick testing to microbial antigens, these reactions, however, did not seem clinically significant [25]. Also among farmers exposed to thyme dust, the relationship between

Table 2. Comparison of farming students reactive to microbial antigens in the test for inhibition of leukocyte migration *versus* non-reactive classmates.

Students' group	Reactive	Non-reactive	p
Number	10	65	
Males, females	6, 4	43, 22	ns
Phadiatop (+)	4 (40.0%)	23 (37.7%)*	ns*
Elevated total IgE	3 (30.0%)	24 (39.3%)*	ns*
Positive SPT	2 (20.0%)	24 (36.9%)	ns
Atopy	4 (40.0%)	23 (35.4%)	ns
Skin diseases	4 (40.0%)	6 (9.2%)	p = 0.009
Upper respiratory diseases	3 (30.0%)	9 (13.8%)	ns
			(p = 0.06)
Lower respiratory diseases	0 (0%)	1 (1.5%)	nt
Work-related symptoms	10 (100.0%)	18 (27.7%)	p = 0.001
Free from major health problems	2 (20.0%)	43 (66.2%)	p = 0.007

p – significance level, nt – not tested because number of observed cases was too low, ns – not significant (p > 0.05 in two-tailed chi-square test), SPT – skin prick tests, * comparison carried out between the 10 reactive and 61 non-reactive students.

cellular reactivity to microbial antigens and skin symptoms could not be confirmed [26]. The results of the present study seem to support the thesis that, in fact, airborne microbes are capable of inducing skin diseases. While carrying out the medical examination, the involved physicians did not know the results of migration inhibition tests. Therefore, the observers' suggestion as a possible bias source can be excluded.

There are observations that aeroallergens known to produce respiratory allergies, may also adhere to the uncovered skin, and are capable of producing allergic skin inflammation in sensitised persons. This process is referred to as airborne dermatitis, and may be caused by a wide range of allergens and irritants [5, 10].

Correlation between cellular sensitivity to airborne microbes and work-related symptoms. Work-related symptoms of various severity were present in all reactive students compared to 27.7% in non-reactive classmates (p = 0.001). Skin symptoms like pruritus, erythema and/or papules were referred by eight reactive students, four students complained of nasal symptoms (nasal blockage, sneezing, nasal discharge) and two students – of cough. In eight of those 10 students, the allergy tests did not reveal any other specific sensitisation than to microbial antigens. The severity of the work-related symptoms varied greatly, but in most cases they were mild.

With respect to the rare occurrence of work-related lung symptoms observed in our work, it is noteworthy that Omland *et al.* [20] in a study of 1,901 Danish farming students, also did not find a significant relationship between occupational exposure and either lung symptoms or lung function.

Discussion of the possible role of airborne bacteria in work-related skin diseases. Little is known of skin symptoms related to bacteria and bacterial products. Most studies of workers exposed to large amounts of airborne bacteria focus on respiratory symptoms [12, 22]. However, Bünger and co-workers [8] have also found a significantly increased frequency of skin diseases among compost workers which, in most cases, was related to increased levels of IgG specific to *Saccharopolyspora rectivirgula* and *Streptomyces thermovulgaris.* Noticeably, also in our group of reactive students, Nos. 6 and 8 (no other possible cause for dermatitis found) had positive reactions to *S. rectivirgula*. Other examples of work-related dermatitis to bacterial products are several reported cases of dermatitis due to *Bacillus thuringiensis* (Bt), widely used as a bacterial insecticide. Commercial *Bt* preparations consist of spores and toxins of this bacterium, there are also trace amounts of viable cells [3, 15, cited after 4]. On plantations extensively sprayed with *Bt* pesticide, Bernstein and colleagues examined 126 agricultural workers and found skin reactions caused by exposure to *B. thuringiensis* in three cases. On skin tests, 50% of the most heavily exposed harvesters showed skin sensitisation to antigens of this bacterium [4]. Interestingly, these authors did not find any *Bt*-related pulmonary symptoms, which is consistent with our data.

Bacterial extracts are potent inducers of immune reactions [27]; this is partly due to the content of superantigens – bacterial products capable of triggering immune reactions in an unspecific way. Superantigens are suspected of playing a role in a variety of skin diseases (discussed in [23]). Moreover, agricultural dust may contain large amounts of bacterial endotoxin, which is also a potent modulator of immunologic response [2, 7, 19]. The main source of endotoxin in agricultural dust is the Gram-negative bacterium *Pantoea agglomerans* [11, 12]. In this aspect it seems interesting that in our group all students who reacted to *P. agglomerans* have had also dermatitis.

Discussion of the possible role of airborne fungi in work-related skin diseases. Of the two students reactive to *Aspergillus fumigatus* (Table 1: Nos. 6 and 7), one was symptom-free and the other had had dermatitis. Allergic dermatitis caused by airborne moulds has been reported only incidentally [14, 16]. However, Bünger and colleagues have found significantly higher concentrations of *A. fumigatus* antibodies among compost workers; in some cases increased IgG level also co-existed with work-related skin disease [8]. Fungal substances are capable of inducing delayed allergic reactions in humans [13]. *A. fumigatus* is a well-known cause of respiratory allergy, which is mostly due to Asp f I/a allergen; it seems, however, that skin reactions are mostly caused by a different antigen of *A. fumigatus* [9].

CONCLUSION

Farming students with cellular reactivity to antigens of airborne microbes suffer more frequently from allergic skin diseases and work-related skin symptoms. This observation encourages further studies of the possible role of environmental microbial antigens in farmers' skin diseases.

Acknowledgements

Completion of this study would not have been possible without the dedicated and skilful work of registered nurses Ms Jolanta Meksuła and Ms Agnieszka Drwal, and the logistic and technical work of Mr Grzegorz Wojciechowski. The research was a part of the Lublin Study (All-Polish Study on Risk Factors for Occupational Allergic Diseases among Farming Students), financed by the Committee for Scientific Research, Warsaw (Grant No. 6 P05D 028 20).

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