

## Significance of IgG antibodies against environmental microbial antigens in a farming population

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### Summary

A group of dairy farmers studied 6 years earlier in a field survey was re-surveyed for respiratory symptoms, occupational capability and the presence of antibodies against environmental micro-organisms. Specific IgG antibodies to *Micropolyspora faeni*, *Thermoactinomyces vulgaris*, *Aspergillus umbrosus* and *Aspergillus fumigatus* were measured by ELISA from the serum samples obtained in the two surveys. Antibody titres remained constant in 70% of both farmers and controls, but where changes took place, the titres against the actinomycete antigens tended to rise, whereas both increases and decreases were detected equally against the *Aspergillus* antigens. The titre of specific antibody to any of the four micro-organisms, when measured from a single serum specimen, seemed to be of little diagnostic value. Observed changes however, were more diagnostic, in that a fall in titre, especially against the *Aspergillus* antigens, was closely associated with a definite decrease in exposure, such as after retirement. Increased titres occurred in farmers with continued exposure, and those against the actinomycetes were associated with the appearance of symptoms in previously symptom-free individuals. In a case of farmer's lung which developed in this population during the follow-up period, significant increases were detectable against *T. vulgaris* and *M. faeni*.

### Introduction

Impurities of environmental air present a major health hazard in many fields of agriculture. Of the resultant occupational diseases, allergic alveolitis is the most serious, even though rare [1]. Other work-related febrile reactions make another distinct disease group associated with a variety of specific working environments [2,3] and chronic bronchitis incipiently forms a greater respiratory problem in agricultural workers than anticipated [4-6].

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Different opinions exist as to the role of antibodies against environmental micro-organisms in the pathogenesis of various environment-associated respiratory disorders. In the case of allergic alveolitis, the importance of certain actinomycetes [7] and possibly fungi [8,9] as inducers of the disease has been established most clearly, but even in this disease the participation of different immune mechanisms and the role of circulating antibodies in the development of the pathological condition have not been documented conclusively [10].

Since Pepys' first description [11], the presence of precipitating antibodies against environmental micro-organisms has been used to detect sensitization in exposed subjects. Double diffusion, the method usually used, is simple, but it is fairly insensitive and poorly quantitative. Newer laboratory techniques, e.g., enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA), first used in infection diagnostics but also found suitable in this field [12,13], offer possibilities for quantitation of specific antibodies. In this study, we evaluated associations between the changes in specific IgG antibody levels against antigens prepared from environmental actinomycetes and fungi as measured by ELISA and the changes in exposure and symptom status of farmers in the light of a 6-year follow-up study. Antigens for testing were extracted from *Micropolyspora faeni* and *Thermoactinomyces vulgaris*, actinomycetes associated with respiratory disease in farmers, and *Aspergillus fumigatus* and *A. umbrosus*, both common fungi in the farming environment in Finland [12].

### Materials and methods

A group of 325 dairy farmers and of 107 controls studied for the presence of respiratory symptoms, and of precipitating antibodies against antigens relevant to the farming environment, were re-studied 6 years after the original field survey by the postal questionnaire previously used for respiratory symptoms and occupational data [4]. The re-survey also included the collection of blood samples for antibody analyses within 6 weeks of receiving the filled questionnaire form. Both surveys took place in January and February to avoid possible seasonal variation which might affect antibody levels. The original control group consisted of rural, non-farming people from the same area as the farmers, matched as a group with the farmers in respect to age, sex and smoking habits. A total of 292 farmers (90%) and 80 controls (71%) were reached for re-evaluation.

#### *Enzyme-linked immunosorbent assay (ELISA)*

Antigens for the ELISA test were extracts prepared from actinomycetes or fungal mycelia as previously described [12]. The microbial strains originated from the Mycological Reference Laboratory, London, except for the *A. umbrosus* strain which was of Finnish origin [14]. Antibody testing was performed according to Voller *et al.* [15] on polystyrene microtitre plates (Dynatech) using peroxidase conjugated anti-human IgG (Orion Diagnostics, Finland). Four ten-fold dilutions of each serum were tested in ELISA. The absorbance curves were calculated on a logarithmic scale of the serum dilutions and the results expressed as titres. A titre was the negative logarithm of the serum dilution giving an absorbance of 0.5 [12].

Serum samples from both surveys were stored at  $-20^{\circ}\text{C}$  without preservatives. The stability of IgG immunoglobulin through the 6-year storage was confirmed by parallel testing of total IgG by the Mancini technique (Behringwerke AG, West Germany) of both samples of each person showing a change in specific IgG against any test antigen.

**Table 1.** Mean titres obtained for the study groups in the primary survey (I) and in the follow-up study (II)

|                     |    | Farmers               |                         | Controls             |                        | Analysis of variance.<br>Significance between<br>groups |
|---------------------|----|-----------------------|-------------------------|----------------------|------------------------|---------------------------------------------------------|
|                     |    | Men<br><i>n</i> = 151 | Women<br><i>n</i> = 138 | Men<br><i>n</i> = 44 | Women<br><i>n</i> = 36 |                                                         |
| <i>M. faeni</i>     | I  | 2.18+0.66             | 2.27+0.64               | 2.19+0.55            | 2.07+0.72              | 0.09                                                    |
|                     | II | 2.19+0.65             | 2.32+0.66               | 2.07+0.65            | 2.17+0.68              | 0.10                                                    |
| <i>T. vulgaris</i>  | I  | 3.38+0.43             | 3.48+0.47               | 3.61+0.53            | 3.51+0.65              | 0.04                                                    |
|                     | II | 3.42+0.49             | 3.49+0.55               | 3.57+0.53            | 3.54+0.63              | 0.33                                                    |
| <i>A. umbrosus</i>  | I  | 1.86+1.02             | 2.36+0.98               | 1.22+0.89            | 1.70+0.65              | <0.001                                                  |
|                     | II | 1.89+1.03             | 2.28+0.94               | 1.15+0.88            | 1.72+0.69              | <0.001                                                  |
| <i>A. fumigatus</i> | I  | 2.36+0.68             | 2.43+0.64               | 2.37+0.57            | 2.34+0.67              | 0.65                                                    |
|                     | II | 2.39+0.74             | 2.44+0.64               | 2.27+0.69            | 2.30+0.65              | 0.47                                                    |

However, no measurable changes in total IgG content were detected. One positive and two negative control sera were included in each series to ensure that the level of titres remained constant throughout testing, the results of each series were adjusted according to the results obtained from the control sera and each series contained a proportionate number of serum samples of both farmers and controls. The two sera of each person were tested in parallel on the same plate and if their titres showed a difference greater than 0.7, or a titre lower than 1.00, the results were confirmed by repeat tests. Unless otherwise mentioned, the Chi-square test was used to test significance in differences between frequencies.

## Results

When measured by IgG ELISA, the degree of sensitization to the four micro-organisms varied greatly in the study groups. Comparing the mean titres, the farming men and women differed significantly from their controls in showing a higher level of sensitization to *A. umbrosus* (Table 1). The farming women also had the highest mean titre against *M. faeni*, but no difference existed between the study groups against *A. fumigatus*. For *T. vulgaris* the highest mean titres were found in the controls; however this difference, more apparent in the first survey, had diminished by the second one. The mean titres against *A. umbrosus* correlated with age, the highest being found in the middle-aged farmers (31–50 years) in the second survey. In the farming men, this correlation by age group was highly significant ( $P < 0.001$ ). Their mean titres ranged from  $2.33 \pm 0.81$  (age group 31–50 years) to  $1.51 \pm 1.01$  (age group > 65 years).

When the level of sensitization was evaluated by the number of persons with high antibody titres in each study group in the primary survey, a similar trend was seen (Table 2). Titres exceeding the 'negative' control serum by 2 s.d. in case of actinomycete antigen or by 3 s.d. in case of *Aspergillus* antigens were considered as high. High antibody titres against *A. umbrosus* and *M. faeni* were more common in the farming men and women than in their controls ( $P < 0.001$  for *A. umbrosus* and  $P < 0.05$  for *M. faeni*), but high titres against *T. vulgaris* were more common in the controls compared to the farmers ( $P < 0.05$ ).

To evaluate the significance of the level of specific IgG antibodies against the test antigens, analysis of variance was used to determine associations of ELISA antibody

levels to various parameters from the questionnaires [4], which included respiratory symptoms and subjective evaluation of mould dust exposure in the previous three years. No clear-cut correlations could be detected, i.e., between the antibody titres and chronic bronchitis or dust-related respiratory symptoms, not even in life-long non-smoking men and women.

Evaluation of individual changes in the levels of antibodies and their correlation to other parameters were also interesting. A difference of 0.7 titre unit (corresponding to a five-fold difference) or greater, in antibody titres between the two sera of the same person was considered as a significant change [12]. The majority of farmers (70%) (Table 3) or controls (74%,  $n=59$ ) had no change in antibody levels against any test antigen. Antibody levels against one or more of the antigens increased in 20% and decreased in 11% of the farmers and in 20% ( $n=16$ ) and 14% ( $n=11$ ) respectively of the controls. Both in the farmers and in the controls, changes in titre were observed twice as frequently in the antibodies against *Aspergillus* spp. as against the actinomycetes. In the farmers, *Aspergillus* antibody changes (increases equally as decreases) were detected in 23% ( $n=68$ ). This contrasted to changes in actinomycete antibodies, detectable in 12% ( $n=35$ ) of the farmers, as these were almost exclusively increases. Increases were detected in 10% ( $n=30$ ), and decreases only in 1% against both *M. faeni* ( $n=4$ ) and *T. vulgaris* ( $n=3$ ). There were also six farmers (2%) who had a rise against one antigen and a fall against another.

In the farmers, decreases in antibody titres were slightly more frequent in the older age groups (Fig. 1) and they were closely related to changes in occupational status ( $P<0.01$ ) (Table 3). The farmers who had retired because of illness showed decreases three times more frequently, and in antibodies against *Aspergillus* antigens four times more frequently than the farmers engaged in farming throughout the follow-up period. The closest association between decreases in *Aspergillus* titre and occupational changes were observed in the middle-aged women ( $P<0.001$ ) (Table 4).

However increases, in contrast to decreases, in antibody levels were more common in farmers continuously exposed to farm dusts (Table 3) and in younger age groups (Fig. 1), especially in the women ( $P<0.05$ ). The link between increases in titre and a history of continued occupational exposure was again most definite in the women

**Table 2.** Prevalence of high antibody titres by study groups in the primary survey

|                                  | Farmers        |                  | Controls      |                 | Significance between groups |
|----------------------------------|----------------|------------------|---------------|-----------------|-----------------------------|
|                                  | men<br>$n=151$ | Women<br>$n=138$ | men<br>$n=44$ | Women<br>$n=36$ |                             |
| High antibody titre against      |                |                  |               |                 |                             |
| <i>A. umbrosus</i> titre > 2.50  | 54<br>36%      | 75<br>54%        | 2<br>5%       | 3<br>8%         | $P<0.001$                   |
| <i>M. faeni</i> titre > 2.80     | 19<br>13%      | 29<br>21%        | 4<br>9%       | 2<br>6%         | $P<0.05$                    |
| <i>A. fumigatus</i> titre > 2.80 | 34<br>22%      | 35<br>25%        | 10<br>23%     | 9<br>25%        | NS                          |
| <i>T. vulgaris</i> titre > 3.90  | 16<br>11%      | 30<br>21%        | 12<br>27%     | 9<br>25%        | $P<0.05$                    |

**Table 3.** Changes in antibody levels associated with changes in occupational status in the farming population

| Occupational data in second survey            | No. changes observed | Decreases           |                    | Increases           |
|-----------------------------------------------|----------------------|---------------------|--------------------|---------------------|
|                                               |                      | against any antigen | against Aspergilli | against any antigen |
| Farming<br><i>n</i> = 208                     | 149<br>72%           | 17*<br>8%           | 12<br>6%           | 45<br>22%           |
| Retired for illness<br><i>n</i> = 42          | 27<br>64%            | 12<br>29%           | 10<br>24%          | 6<br>14%            |
| Changed occup. or on pension<br><i>n</i> = 41 | 29<br>71%            | 4<br>10%            | 4<br>10%           | 8<br>20%            |
| Total number<br><i>n</i> = 291                | 205<br>70%           | 33<br>11%           | 26                 | 59<br>20%           |
|                                               |                      | $P < 0.01^{**}$     |                    | $P = 0.05^{**}$     |

\* Six farmers had both increases and decreases against different organisms.

\*\* Significance between groups with different occupational status.

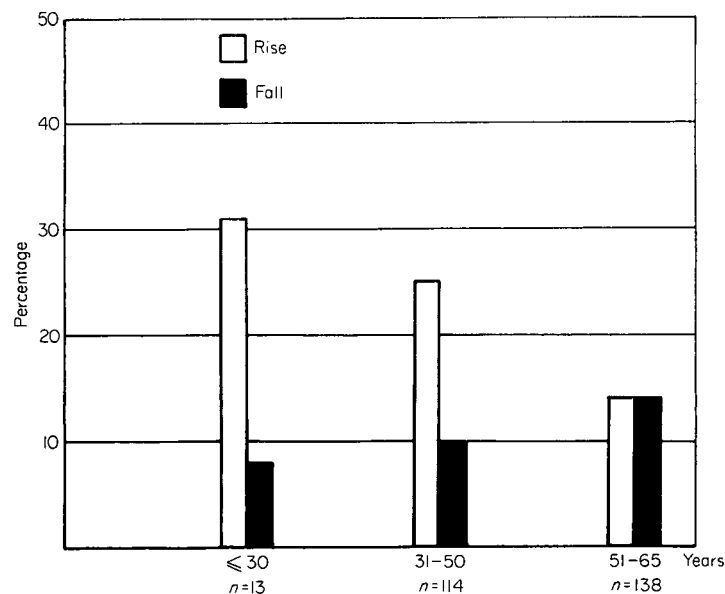
**Fig. 1.** Increases and decreases in IgG antibody titres by age groups in the working farmers.

**Table 4.** Association of changes in antibody titre and changes in occupational status in women aged 30–65 years in the second survey

| Occupational data in second survey  | <i>Aspergillus</i> titre |          | <i>M. faeni</i> titre |         | <i>T. vulgaris</i> titre |         | Any titre    |           |
|-------------------------------------|--------------------------|----------|-----------------------|---------|--------------------------|---------|--------------|-----------|
|                                     | Rise                     | Fall     | Rise                  | Fall    | Rise                     | Fall    | Rise         | Fall      |
| Farming (n=90)                      | 12<br>13%                | 3<br>3%  | 11<br>12%             | 2<br>2% | 7<br>8%                  | 2<br>2% | 22<br>24%    | 6<br>7%   |
| Retired for illness (n=16)          | 0<br>0%                  | 6<br>38% | 1<br>6%               | 0<br>0% | 0<br>0%                  | 0<br>0% | 1<br>6%      | 6<br>38%  |
| Changed occup. or on pension (n=13) | 0<br>0%                  | 2<br>15% | 0<br>0%               | 0<br>0% | 0<br>0%                  | 1<br>8% | 0<br>0%      | 2<br>14%  |
| Total number (n=119)                | 12<br>10%                | 11<br>9% | 12<br>10%             | 2<br>2% | 7<br>6%                  | 3<br>3% | 23<br>20%    | 14<br>12% |
|                                     | $P < 0.001^*$            |          | NS*                   |         | NS*                      |         | $P < 0.01^*$ |           |

\* Significance between groups with different occupational status.

( $P < 0.01$ ) (Table 4), twenty-two of the twenty-three detected increases being found in the women remaining in farm work.

Associations between changes in respiratory symptom status and changes in antibody levels were further studied in the group of farmers who continued farming throughout the follow-up period. For every third farmer, and for every second woman, who had developed respiratory symptoms during the follow-up a rise in antibody level was found against *M. faeni* and/or *T. vulgaris* and/or *A. fumigatus*, whereas in asymptomatic farmers increases were detected in approximately 10% (Table 5). In the women, six of the fourteen (43%) newly symptomatic had increases in titre compared to seven of the sixty (12%) without any symptoms, and to five out of the twenty-four (21%) with symptoms in both surveys. Despite the small number of newly symptomatic women in the study group, the observation was statistically close to the significance level ( $P = 0.08$ ). Contrary to the clear association between changes in exposure status and antibody levels against *A. umbrosus*, changes in *A. umbrosus* antibody levels lacked any correlation to respiratory symptoms in the farmers with continued exposure.

In this group of farmers, there was one woman who developed acute farmer's lung during the follow-up period, for which she was hospitalized. In the first survey, she had positive precipitins against *T. vulgaris* but negative against *M. faeni* and *A. fumigatus*. Having developed farmer's lung, her antibody titres against *T. vulgaris* increased during the follow-up by 1.93 and against *M. faeni* by 0.95, both of which were significant increases.

## Discussion

Earlier studies on farmer's lung disease have revealed that *A. umbrosus*, *T. vulgaris* [14], as well as *A. fumigatus* [16] are common sensitizers in patients with farmer's lung in Finland. Antibodies against *M. faeni*, the micro-organism most commonly associated with this disease, are of minor importance in these patients [14, 16] and also *M. faeni* is rare in agricultural dusts in the same area [14]. In the present study of a working exposed population and not patients, antibodies against the test micro-organisms were

**Table 5.** Association of respiratory symptoms including dust related symptoms and chronic bronchitis and changes in antibody titres against *Micropolyspora faeni*, *Thermoactinomyces vulgaris* and *Aspergillus fumigatus* in person remaining in farm work to the end of the follow-up period

| Change in antibody titre against any microbe | Symptom status                          |                          |                              |          |
|----------------------------------------------|-----------------------------------------|--------------------------|------------------------------|----------|
|                                              | Appearance of symptoms by second survey | Symptoms in both surveys | No symptoms in second survey |          |
|                                              |                                         |                          | Number                       |          |
| Increased titre                              | 8 (29%)                                 | 11 (17%)                 | 12 (10%)                     | 31 (15%) |
| Decreased titre                              | 0 (0%)                                  | 5 (8%)                   | 4 (3%)                       | 9 (4%)   |
| Total number of persons in each group        | 28                                      | 66                       | 118                          | 212      |

sign  $P=0.07$

commonly found in farmers but some were also found in a non-farming rural population without known occupational exposure to any agricultural dusts. Although some in the control group had a past history of work on farms, it mostly dated back to adolescence, and a minimum of 4 years away from farm work had been required for acceptance into this group. In an earlier publication [16] it was also shown that a previous farming history has no effect on the prevalence of precipitating antibodies against these micro-organisms in unexposed rural persons.

By analysis of the antibody responses, *A. umbrosus* and *M. faeni* seemed to be most closely associated with farm dust exposure, whereas *T. vulgaris* was found mainly in non-agricultural environments and *A. fumigatus* commonly in all rural environments. Within the farming population, women had higher antibody titres than men, reflecting their work role in the cattle barns. Earlier studies in Finland have also shown both precipitin findings [4] and patients with farmer's lung [1] to be more frequent in farming women than men. In the control group, no sex-linked association to high antibody titres was observed.

The study design excluded a possible seasonal variation in the specific IgG antibody levels which might be present in persons exposed to mould dusts in varying degrees during indoor and outdoor feeding seasons. Both surveys took place in January and February. Individual changes in antibody titre between the serum samples taken 6 years apart were surprisingly uncommon, the majority of persons having no significant variation in their specific antibody levels. Antibodies against the two *Aspergillus* spp. and the two actinomycetes, showed somewhat varying patterns in the follow-up period. There were twice as many changes in antibody levels against the *Aspergillus* antigens as against the actinomycetes, and both increases and decreases were equally common. Changes in titre against the actinomycetes were fewer and mainly increases were observed. Reasons for this difference might be a greater year-to-year variation in the amount of *Aspergillus* compared to thermophilic actinomycetes, or a more stable antibody response to the actinomycete antigens. Once antibody to either one of the actinomycetes was detected, no decrease in titre would be expected, since decreases were observed in only 1% of the farmers, whereas increases were found in 10%.

The level of antibody detected in this study population was not comparable to

those high levels frequently found by the same method in patients with farmer's lung disease [12]. In this 'healthy' farming population, decreases in antibody titres were found mostly following definite decreases in exposure, as in retirement caused by illness, although this was not a rule. In the women, who generally had higher levels of antibody as well as higher levels of dust exposure because of their work in cattle barns, associations between antibody changes and various working parameters were clearer. In *Aspergillus* antibodies, especially against *A. umbrosus*, this 'ceasing of exposure-fall in titre' association was most clearly seen in the women, whilst in the men antibody levels, particularly to the *Aspergillus* antigens, displayed both increases and decreases, even in those retired. In many respects, microbial antibodies behave in a controversial manner in men and women both when measured by precipitation [4] or as here when measured by ELISA, and associations to different parameters seldom show similar features. This discrepancy between men and women probably lies both on the different working habits of men and women even on small farms and on their different smoking habits. The majority of men (67%) were smokers or ex-smokers whereas only 9% of the women had ever smoked. Men retiring from their usual field work because of incapacity may be even more exposed to barn dust than before. When women become unable to work on their farm they turn to indoor household work, thus totally avoiding farm dust exposure. The effect of smoking on immunological responses, on the other hand, is still very poorly defined, although some evidence suggests it to be of suppressive nature [17].

Increases in antibody titres were more common in the age groups with most efficient working capacity. In the women, who seldom were smokers and thus not expected to have smoking-associated respiratory symptoms, there seemed to be a trend for increases in antibody level to develop, especially in those who had started to experience respiratory symptoms during the follow-up period. This could either be a sign of a heavy dust load, which had simultaneously induced both symptoms and antibody response, or a sign of antibody mediation for the symptoms. However the symptoms registered were not those particularly suggesting farmer's lung but rather chronic bronchitis or other symptoms involving the bronchial tree. Interestingly, the new acute case of farmer's lung which appeared in this study population during the follow-up was associated with definite increases in antibody to both of the actinomycetes, but lacking any change to the *Aspergillus* antigens. The incidence rate of one new case of farmer's lung in a group of 325 per 6 years is what could be expected in Finland [1].

It can be concluded from this study that in population surveys one single determination of the antibody level against any of the four microbial antigens is not by itself diagnostic. The situation is different in clinical cases of farmer's lung [13] as well as in pigeon breeder's disease [18], another form of allergic alveolitis, where a correlation between the antibody level and the presence of symptoms is much clearer. However, as in infection diagnostics, changes in antibody titre are more valuable than single serum determinations. Decreases are closely related to definite decreased exposure, and an observation of an increase in antibody titre to microbial antigens may lead to the tracing of the inducer of respiratory symptoms, even in chronic bronchitis. As in infection diagnostics, a recent preliminary study has shown that the detection of class or sub-class specific antibodies, in farmer's lung of IgG, IgA and IgG3, may be of importance in clinical diagnosis (Ojanen *et al.*, submitted).

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