Class-specific antibodies during follow up of patients with farmer’s lung


Abstract: Sequential serum samples of 13 patients with acute farmer’s lung (FL) taken during a follow-up of 18-36 months, were tested for antibodies of immunoglobulin G (IgG), IgA, IgM and IgE classes against Thermotomycyes vulgaris and Micropolyspora faeni, and compared with contemporary lung function parameters. In the acute phase, antibodies of several Ig classes were present, those of IgG and IgA being most common. At the end of the follow-up, the mean values of all antibody titres were lower than in the acute phase, and antibodies were mostly of one or two Ig classes only. The reduction in antibody levels was most often detectable in IgG and IgA antibodies against T. vulgaris. Antibody titres correlated inversely with tested lung function parameters, especially IgA antibodies with pulmonary diffusing capacity. Our results show that a follow-up of class-specific antibodies, especially of IgG and IgA, gives valuable information on causative microbes and on temporal changes of the exposure.


In the diagnosis of farmer’s lung (FL), antibodies against environmental microbial antigens provide useful evidence of exposure to possible causative agents. Enzyme-linked immunosorbent assay (ELISA) is increasingly used to test the sera for antibodies. Among other advantages, ELISA offers the possibility to measure antibodies of different immunoglobulin (Ig) classes. With ELISA, IgG antibodies against the actinomycetes Micropolyspora faeni [1] and Thermotomycyes vulgaris [2], and IgG, IgA, IgM and IgE antibodies against M. faeni have been measured in FL patients [3]. FL patients are usually tested for antibodies of IgG during an acute attack of the disease. Little information is available on the dynamics of antibody levels after the acute phase and on the level of other Ig classes. This kind of information would be valuable for assessing the diagnostic significance of antibody tests.

In the present study, serial determinations of antibodies of FL patients were performed during a follow-up period of 18-36 months after an acute phase. Antibodies of different Ig classes against the actinomycetes T. vulgaris and M. faeni, which are considered the main causative microbes [4] in FL, were measured. The association between antibodies and the disease was estimated by comparing antibody levels with lung function parameters.

Patients

The study group included 9 women and 4 men. Diagnosis of FL disease was confirmed on the basis of symptoms and signs, changes in chest roentgenograms and physiological findings [5]. The age of the patients was 29-62 yrs. Only one was a smoker. The patients were hospitalized because of a first acute episode of FL disease. During a follow-up only one of the 13 patients had another acute attack. Lung function parameters were measured during hospitalization and subsequently 5-7 times during a follow-up period which was 18 months for five patients, 24 months for seven and 36 months for one patient. On each occasion serum samples were obtained from each patient. After hospitalization patients continued to work in their farms but were advised to use dust respirators when handling hay or straw.

Physiological tests

Several lung function parameters were measured during follow-up, including pulmonary diffusing capacity for carbon monoxide (DLCO), carbon monoxide transfer coefficient (Kco) and arterial oxygen tension (PaO₂) at rest. These parameters were chosen because...
they were considered to reflect the changes on the alveolar level best. DLCO was measured using a Morgan Respirometer Mark 4 (P.K. Morgan Ltd, London, UK) and Pao2, using a Clark-type oxygen electrode, type E 5046 (Radiometer A/S, Copenhagen, Denmark).

Methods

Enzyme-linked immunosorbent assay

Antigens for antibody determinations. Mycelial antigens were prepared from T. vulgaris and M. faeni (Mycological Reference Laboratory, London, UK) as described previously [2]. Microbes were grown in nutrient broth (0.5% peptone, 0.3% beef extract, Difco) at 55°C for 3–4 days. The cells were harvested by centrifugation and suspended in 0.05 M phosphate buffered saline (PBS), pH 6.8. The actinomycetes were disrupted by ultrasonic treatment. After low speed centrifugation the supernatants were used as antigens in ELISA.

Antibody determinations. ELISA used to test sera for antibodies of IgG, IgA, IgM and IgE against the two FL microbes was performed on microtitre plates as described previously [2]. IgG and IgM antibodies against both actinomycetes and IgA antibodies against M. Faeni were measured using peroxidase-conjugated anti-human IgG, IgM or IgA (Orion Diagnostica, Finland). The other antibodies were measured by an indirect modification of ELISA. Patient serum was followed by rabbit anti-human IgA (Miles-Yeda, Israel) or IgE (Dakopatts, Denmark). The conjugate was peroxidase-conjugated anti-rabbit IgG (Orion Diagnostica, Finland). ELISA results were expressed as titres. The titre of the serum was the negative logarithm of the serum dilution that gave an absorbance of 0.5. In each series there was a negative reference serum which consisted of pooled sera from healthy controls. A serum was considered positive for antibodies if the titre of the serum exceeded that of the negative reference serum by two standard deviations. A change of 0.5 in antibody titres was considered significant.

Statistical analysis

The titres of different antibody classes and the antibody titres and lung function parameters of individual patients were compared by Kendall’s correlation test. Pearson’s correlation test was used to compare the antibody titres and lung function parameters of the whole study group. Paired sample t-test was used to compare results obtained in the acute phase with results at the end of follow-up.

Results

The prevalence of class-specific antibodies in the acute phase of FL and at the end of follow-up is shown in table 1. In the acute phase antibodies of IgG were usually detected simultaneously with those of IgA or IgE. At the end of follow-up patients were positive for antibodies of fewer Ig classes than in the acute phase.

The measurements for antibodies of all four Ig classes of 13 patients yielded a total of 52 values of antibody titres for both microbes at each sampling time. When the antibody titres were analysed by time, significant decreases, 0.5 titre units or more, were found in antibody levels of some patients after only three months. The number of decreasing antibody titres increased by time (table 2). Both antibody titres considered positive and negative were observed to decrease during a follow-up. A decrease was most often observed in IgG and IgA antibodies. Decreases were detected in T. vulgaris antibodies of 11 patients and in M. faeni antibodies of 9 patients. Among the 52 values for T. vulgaris antibodies 27 titre decreases (52%), in M. faeni antibodies there were 16 decreases (31%). At the end of follow-up period of 18–36 months the mean titres of all antibodies were significantly lower than in the acute phase (table 3). Even if antibody titres were gradually decreasing they did not always reach the level regarded as negative and after the follow-up most patients could still be considered positive for antibodies (table 1).

In the acute phase of the disease lung function parameters of all patients were below normal. During the

<table>
<thead>
<tr>
<th>Microbe</th>
<th>G</th>
<th>A</th>
<th>G+A</th>
<th>G+E</th>
<th>A+E</th>
<th>G+A+E</th>
<th>G+A+M+E</th>
<th>any</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute phase</td>
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<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>at the end</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>acute phase</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
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<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>at the end</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

FL: farmer’s lung; Ig: immunoglobulin.
follow-up lung function parameters gradually improved and did not reach reference values. At the end of the follow-up, the mean values of DLCo (8.8 mmol·min⁻¹·liters⁻¹) and Pao₂ (12.3 kPa) were significantly higher than at the acute phase (6.0 and 10.3, respectively). For DLCo of each patient was calculated as a percentage of the predicted value as described by Viljanen et al. [6]. Antibodies of IgA against both actinomycetes showed the highest correlations with the corrected DLCo (0.001 <p<0.01). With other antibody classes significant correlations were not obtained.

### Table 4. Number of patients with significant correlations (Kendall's correlation coefficient; 0.001 <p<0.01) between antibody titres and DLCo

<table>
<thead>
<tr>
<th>Antibody class</th>
<th>Microbe</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. vulgaris</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M. faeni</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Ig: immunoglobulin; DLCo: pulmonary diffusing capacity for carbon monoxide.

![Fig 1. An example of the pattern of relations between antibody titres and lung function parameters during the follow-up where all antibody titres are inversely correlated with DLCo. (DLCo=mmol·min⁻¹·liters⁻¹). DLCo: pulmonary diffusing capacity for carbon monoxide; Ig: immunoglobulin; ELISA: enzyme-linked immunosorbent assay.](image)

### Discussion

In the present study, the most frequently detected antibodies were those of the IgG and IgA class. A similar observation was made by Patterson et al. [7] for antibodies of T. vulgaris and M. faeni. In the acute phase most of the FL patients in our study had antibodies of several Ig classes. This finding is similar to that of Gari et al. [8], who found exposed but asymptomatic subjects had antibodies of IgG class whereas FL patients had antibodies of IgG and other Ig classes. During a follow-up period levels of antibodies decreased. At the end of follow-up most of our patients were still positive for antibodies but of one or two Ig classes only. This may be typical for FL patients or different microbes may induce immune response predominantly in certain Ig classes. T. vulgaris may induce antibodies of several Ig classes better than M. faeni.
After the acute phase of the disease and hospitalization, our FL patients returned to their normal home and working environment. They were advised to continue their previous farming tasks, but always to use effective dust respirators when handling hay or straw. It was not possible to control the use of dust respirators and it is, thus, difficult to estimate to what extent they were still exposed to FL microbes. Serial antibody analysis of the patients revealed a complex pattern of antibody titres. Some antibody classes decreased while others remained constant. At the end of follow-up the mean titres of all antibodies were significantly lower than in the acute phase indicating a diminished microbial exposure. Antibody titres which most clearly decreased were those against \textit{T. vulgaris}. This supports the role of \textit{T. vulgaris} as a causative microbe of FL or at least as the main sensitizing microbe.

Lung functions measured during the follow-up visits were considered to serve as physiological indicators of disease. Comparison of lung function parameters and the levels of antibodies of different Ig classes should help to assess the diagnostic value of class specific antibodies. In this study we were able to find correlations between some of the lung function parameters and antibodies. In five patients such correlations were not observed even if patients were considered positive for antibodies. This may indicate that in these cases other microbes, not tested here, could be responsible for FL disease. Behaviour of antibodies of \textit{T. vulgaris} supported the role of \textit{T. vulgaris} as an important causative agent of FL. In another allergic alveolitis, pigeon breeder's disease levels of specific IgG antibodies have been shown to correlate with symptoms of disease [9]. When antibody titres and diffusing capacities, expressed as percentage of the predictive value, were analysed for all patients as a group, only IgA antibodies of \textit{T. vulgaris} and \textit{M. faeni} showed a significant inverse correlation with the lung function. Correlation has previously been found between antibodies of IgG sublass and lung function [10]. It may be relevant to these observations that one common property of IgG and IgA is their short half-life in serum. Antibodies with short half-lives may reflect exposure more faithfully than those with a long half-life.

Recent studies have emphasized the value of bronchoalveolar lavage in the diagnosis of FL [11]. However, a serum sample can be obtained more easily and, therefore, any improvement in the serodiagnosis of FL is important. Based on our present study, it seems that testing sera for antibodies of several Ig classes, at least for both IgG and IgA, gives more accurate information on continued or recent exposure and on causative microbes than testing sera only for precipitins or for microbe-specific IgG antibodies.

References


RÉSUMÉ: Des échantillons sériels de sérum de 13 patients atteints de poumon de fermier, pris au cours d'un suivi de 18 à 36 mois, ont été testés pour les anticorps des classes IgG, IgA, IgM et IgE contre deux antécaires. \textit{Thermobacterium vulgaris} et \textit{Microsporum gypseum}, ont été comparés avec les paramètres fonctionnels pulmonaires contemporains. Dans la période aiguë de la maladie, tous les patients ont des anticorps contre l'un ou l'autre des deux germes. L'on observe habituellement des anticorps de plusieurs classes d'immunoglobulines, ceux appartenant aux classes IgG et IgA étant les plus courants. A la fin du suivi, les valeurs moyennes de tous les titres d'anticorps sont significativement plus faibles que dans la phase aiguë. Les anticorps apparaissent le plus souvent à une ou deux classes d'immunoglobulines seulement. Une diminution des anticorps spécifiques de différentes classes d'immunoglobulines a été détectée dans 41% des cas. Une diminution de l'immunoglobuline n'est pas significative pour les anticorps IgG et IgE contre \textit{T. vulgaris}. Les titres d'anticorps IgG et IgE contre \textit{T. vulgaris} et \textit{M. faeni} sont inférieurs à ceux observés dans les groupes de patients atteints de poumon de fermier. Le pourcentage de la valeur prédictive. Nos résultats soulignent la signification de \textit{T. vulgaris}, plutôt que de \textit{M. faeni}, comme agent causal du poumon de fermier en Finlande. Le suivi quantitatif des anticorps de différentes classes d'immunoglobulines, et en particulier celui des IgG et des IgA, donne des informations utiles sur l'exposition persistante récente aux agents provocateurs.