Class specific antibodies in serodiagnosis of farmer's lung

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Abstract

The aim of the present study was to determine which microbes and which immunoglobulin (Ig) classes should be included in tests to discriminate between patients with farmer's lung and reference persons. The sera of a group of farmer's lung patients and their spouses were measured for IgG, IgA, IgM, and IgE antibodies against a panel of farmer's lung microbes. The concentrations of IgG, IgA, and IgE antibodies were higher in patients compared with their spouses. The patients were generally positive for antibodies of several Ig classes whereas the spouses had only either IgG or IgA antibodies. A test comprising the determinations of IgG antibodies against *T vulgaris* and IgA antibodies against *A fumigatus* would correctly group 94% of the cases in the Finnish farming population. The selection of microbes for other environments needs to be determined locally.

The diagnosis of farmer's lung disease is based on a set of criteria.^{1,2} One of the criteria is the presence of antibodies against microbial antigens. Antibodies against microbes associated with farmer's lung are more common and their concentrations are higher in farmer's lung patients than in healthy reference persons.³⁻⁵ Some of the bacteria and fungi involved in farmer's lung, however, are common environmental microbes, and antibodies can be detected also in symptomless healthy persons. Presence of antibodies against these microbes alone is therefore not diagnostic for farmer's lung disease. Development of laboratory methods that would help to identify clinically significant antibody profiles is of great importance. The adaptation of enzyme linked immunosorbent assay (ELISA) to measure antibodies in farmer's lung⁴⁻⁶ has made it possible to measure accurately and quantitatively antibodies of different immunoglobulin (Ig) classes.

The aim of the present study was to determine

which antibody classes and which microbes should be included in the diagnostic test panel of farmer's lung for optimal results. Enzyme linked immunosorbent assays were used to test the sera for antibodies of IgG, IgA, IgM, and IgE against *Aspergillus umbrosus*, *Aspergillus fumigatus*, *Thermoactinomyces vulgaris*, and *Micropolyspora faeni* (*Facnia rectivirgula*), the microbes most commonly associated with farmer's lung in Finland.

Material

The study group included 24 farmer's lung patients, 18 women and six men, and the reference group comprised their spouses.^{7,8} The farmer's lung patients were admitted to hospital because of an acute episode of the disease. Diagnosis of farmer's lung was based on the criteria formulated by Terho.² The main criteria were: exposure to offending antigens, symptoms compatible with farmer's lung³ present and appearing or worsening some hours after exposure to antigen and lung infiltration compatible with farmer's lung.⁹ The additional criteria were: basal crepitant rales audible on auscultation of the lungs, impairment of the pulmonary diffusing capacity, oxygen tension of arterial blood either decreased at rest or normal at rest but decreased during exercise, restrictive ventilation defect in spirometry, histological changes compatible with farmer's lung in the biopsy specimen from the lung, and a positive provocation test either by work exposure or by controlled inhalation challenge. The diagnosis was made when the patient fulfilled all the main criteria and at least two of the additional criteria. The spouses were also working on farms under the same microbial exposure as the patients. Table 1 presents basic information of the study and reference groups obtained from patient records and by postal inquiry. The subject was considered to have symptoms of farmer's lung if she or he had cough and respiratory distress or fever after some hours of having handled mouldy hay or other mouldy material. Blood samples were obtained from each patient the day after admission to hospital. Blood samples of the spouses were collected subsequent to admission of the patient to hospital, during the same indoor cattle feeding season.

Antigens

Mycelial antigens were prepared from *Aspergillus*

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Table 1 Age and anamnestic data of the patients (n = 24) and spouses (n = 24)

	Number of persons reporting				
	Age (y (SD)) (Range)	Smoking*	Exposure†	Recent exposure‡	Symptoms§
Patients	48 (10) (29-69)	1	22	19	19
Spouses	50 (9) (30-67)	8	24	18	3

*Current smokers and ex-smokers with less than six months of non-smoking.

†Handling of mouldy hay or other mouldy material within past five years.

‡Handling of mouldy hay or other mouldy material during past two months.

§Cough and respiratory distress or fever after handling mouldy material during past two years.

umbrosus (isolated from Finnish mouldy hay),¹⁰ *Aspergillus fumigatus* (2140), *Thermoactinomyces vulgaris* (1156) and *Micropolyspora faeni* (1150) (Mycological Reference Laboratory, London, UK) as described previously.^{5,11} *A. umbrosus* was grown in synthetic histoplasmin medium supplemented with 10% NaCl at room temperature for three to four weeks. *A. fumigatus* was grown in nutrient broth (0.5% peptone and 0.3% beef extract, Difco) at 37°C for two days. The actinomycetes were grown in nutrient broth at 56°C for three to four days. Microbial cells were harvested by centrifugation and suspended in 0.05 M phosphate buffered saline (PBS), pH 6.8. The fungi were disrupted mechanically (Ultra Turrax) and then by ultrasonic treatment and the actinomycetes by ultrasonic treatment only. After low speed centrifugation the supernatants were lyophilised. For reconstitution, water was added to a protein concentration of 1 mg/ml. After dilution to 1:1000 in carbonate buffer they were used as antigens to coat microtitre plates.

Methods

ANTIBODY DETERMINATIONS

The ELISA, used to test sera for antibodies of IgG, IgA, IgM, and IgE, was performed on microtitre plates as described earlier.⁵ Titres of IgG, IgA, and IgM antibodies were measured using peroxidase conjugated antihuman IgG, IgA, or IgM (Orion Diagnostica, Finland); IgE antibodies were measured by an indirect modification of ELISA. Serum to be tested was followed by rabbit antihuman IgE (Miles Yeda, Israel). The conjugate was peroxidase conjugated antirabbit IgG (Orion Diagnostica, Finland). The absorbances at 492 nm were measured by Titertek Multiscan (LabSystems, Finland). The 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 that consisted of pooled sera from healthy students. Before sera were pooled their absorbances were measured and the means and standard deviations were calculated. A serum to be tested was considered positive for antibodies if the titre of the serum exceeded the mean value of the negative reference

sera by two standard deviations. The antibody titres of the two test groups were compared with Student's *t* test and stepwise discrimination analysis with Wilks' lambda using the SPSSPC+ program.

Results

Tables 2 and 3 present the mean antibody titres of patients and spouses against farmer's lung microbes. Antibody titres of IgG, IgA, and IgE against fungi were significantly higher in the patients than in the spouses. There was no difference in the titre of IgM antibodies. Antibodies against *T. vulgaris* differed significantly in all Ig classes but those against *M. faeni*

Table 2 Antibody titres of farmer's lung patients (n = 24) and their spouses (n = 24) against fungal antigens

	Antibody titres			
	Patients (Mean (SD))	Spouses (Mean (SD))	t Value	p Value
<i>A. umbrosus</i> :				
IgG	3.9 (0.7)	2.5 (1.1)	5.40	<0.001
IgA	1.8 (1.0)	1.0 (0.7)	3.23	<0.01
IgM	2.1 (0.8)	1.9 (0.8)	0.83	NS
IgEx	3.0 (0.5)	2.1 (0.6)	2.98	<0.01
<i>A. fumigatus</i> :				
IgG	3.1 (0.6)	2.3 (0.7)	4.50	<0.001
IgA	1.4 (1.1)	0.4 (0.5)	3.72	<0.001
IgM	1.5 (0.9)	1.5 (0.9)	0.14	NS
IgE*	1.8 (0.8)	0.9 (0.7)	2.88	<0.01

*n = 18

Table 3 Antibody titres of farmer's lung patients (n = 24) and their spouses (n = 24) against actinomycetes

	Antibody titres			
	Patients (Mean (SD))	Spouses (Mean (SD))	t Value	p Value
<i>T. vulgaris</i> :				
IgG	4.6 (0.8)	3.0 (0.5)	8.57	<0.001
IgA	2.4 (1.0)	1.1 (0.7)	5.06	<0.001
IgM	2.3 (0.9)	1.2 (0.9)	4.46	<0.001
IgEx	3.5 (0.5)	2.4 (0.6)	5.86	<0.001
<i>M. faeni</i> :				
IgG	3.8 (0.7)	3.2 (0.4)	3.23	<0.01
IgA	2.0 (0.7)	1.6 (0.4)	2.17	<0.05
IgM	2.6 (0.5)	2.2 (0.8)	2.17	<0.05
IgE*	Not tested			

*n = 18

Prevalence of class specific antibodies in farmer's lung patients and their spouses. Only antibodies with significant differences between the study group are presented.

differed only in IgG antibodies. The exclusion of the titres of smokers or the titres of spouses with symptoms of farmer's lung (and of the corresponding patients) had no effect on the means of titres or values of *t*.

The difference between the patients and their spouses was also clear when the prevalences of positive antibody reactions were compared. This is shown in the figure, in which the prevalences of antibodies with significant differences in mean titres between the groups (tables 2 and 3) have been drawn. Antibodies of the IgG and IgA classes were most common in both study groups; IgM and IgE antibodies were found only in patients.

When the results for each person were analysed individually by microbe and number of Ig classes found positive, the patients were generally positive in antibodies of two or more Ig classes whereas spouses had antibodies of one Ig class only. Table 4 shows the occurrence of the most common combinations. Against the fungi, the most common combination is the presence of IgG and IgA antibodies against *T. vulgaris*; patients were positive in three or all four Ig classes (three patients). Only two spouses in the study group were positive for both IgG and IgA antibodies.

For a more detailed evaluation of the ability of different antibody determinations to distinguish

between the patients and the spouses, the stepwise discriminant analysis method was used. The results show that antibodies against *T. vulgaris* could discriminate the patients from the spouses best. When the same analysis was performed on different Ig classes, IgG turned out to be the most important and the addition of IgA or IgE antibodies against the same microbe only slightly improved the percentage of correct classifications.

Table 5 shows the results when both the microbe and the Ig class were taken into account. As could be expected IgG antibodies against *T. vulgaris* gave the best distinction between the patients and the healthy spouses. A panel consisting of IgG antibodies against *T. vulgaris* and IgA antibodies against *A. fumigatus* classified the patients and the spouses correctly in 94% of the cases, with a coefficient of determination of 72%. The addition of *M. faeni* and *A. umbrus* to the panel only slightly improved the discrimination between the study groups. When the titres of spouses with symptoms of farmer's lung and those of the corresponding patients were excluded from the analysis the results remained essentially the same.

Discussion

Serological diagnosis in farmer's lung depends on several factors. The choice of microbial antigens is

Table 4 Number of patients and spouses with positive antibody titres of different Ig classes against farmer's lung microbes

Positive antibody titres	<i>A umbrosus</i>		<i>A fumigatus</i>		<i>T vulgaris</i>		<i>M faeni</i>	
	P	S	P	S	P	S	P	S
IgG only	6	1	5	3	5	1	12	8
IgA only	3	1	4	4	0	1	1	0
IgG + IgA	9	2	8	0	4	0	4	0
IgG + IgE	1	0	0	0	0	0	0	0
IgG + IgA + IgE	3	0	2	0	8	0	0	0
IgG + IgA + IgM	0	0	0	0	0	0	1	0
IgG + IgA + IgM + IgE*	0	0	0	0	3	0	0	0
Any	22	4	19	7	20	2	18	8

P = Patients (n = 24); S = spouses (n = 18). *n = 18

Table 5 Different microbe and class specific antibody combinations entered in stepwise discriminant analysis to find the best discriminating combination

Step	Microbe	Ig class	Coefficient of determination (cumulative %)	% Of grouped cases correctly classified (cumulative)
1	<i>T vulgaris</i>	IgG	61	90
2	<i>A fumigatus</i>	IgA	72	94
3	<i>T vulgaris</i>	IgM	76	94
4	<i>M faeni</i>	IgA	79	94
5	<i>A umbrosus</i>	IgE*	81	96

*n = 18

the first to be considered. Local surveys of the environmental microbial flora and antibody screenings are prerequisites for the selection of representative microbes in the antigen panel. According to this and to a previous study,⁸ *T vulgaris* seems to be the microbe most closely associated with farmer's lung in Finland. *T vulgaris* was also the predominant actinomycete in the air of Finnish farms.¹² In other countries the situation may be different.¹³ Our comparative study on farmer's lung patients from four countries confirmed the importance of *M faeni* in the United States and *T vulgaris* in Finland. Therefore microbes for an antigen panel ought to be selected individually for each region. The choice of immunological method to test antibodies is also to be considered. The ELISA appears to be the method of choice because it enables the determination of class specific antibodies that seem to have diagnostic significance. Preparation of antigens is dependent on the method of determination. Extracts of microbial cells are suitable for ELISA but in gel precipitation concentrated culture fluids are better.¹¹ Antigens used today are crude microbial extracts or culture filtrates, but for the development of ELISA tests, purification and standardisation of antigens are important goals.¹⁴

In farmer's lung, the significance of antibodies against different microbes is evaluated by comparing the antibody titres between groups, selected by a range of criteria. In this study, antibody titres of farmer's lung patients were compared with those of their spouses. Spouses were selected as reference

persons because they lived and worked in the same environment as the patients and were exposed to the same microbial antigens but had not developed clinically diagnosed farmer's lung even though three of the spouses reported symptoms suggestive of farmer's lung in the questionnaire. As women are most often affected by farmer's lung in Finland this leads unavoidably to a predominance of men and smokers in the group of spouses. Smoking is one of the factors that has been shown to depress the development of antibodies of both IgG and IgA classes.¹⁵⁻¹⁷ In this study population, however, mean antibody titres and differences in antibody titres between the groups were not influenced by smoking habits.

It is typical of the acute phase of farmer's lung that patients often have antibodies of several Ig classes against several microbes¹⁸ by contrast with control persons who have only IgG antibodies against one or two microbes.¹⁹ According to the previous study,⁸ after an acute phase, patients gradually become negative in some of the Ig classes. This is seen already after three months but is most prominent after a longer period. In this study acute phase sera of patients were measured. Thus most of the patients were positive for antibodies of several Ig classes whereas the spouses generally had antibodies of one Ig class only, either IgG or IgA. Only two of the spouses had both IgG and IgA antibodies against *A umbrosus* and the IgA antibodies were only just above the limit considered positive.

The present study was designed to develop the

optimal panel for serodiagnosis of farmer's lung in Finland. The results in table 5 provide a basis for the selection. According to the results it can be suggested that for the serological diagnosis of farmer's lung in Finland, the test should include IgG antibodies against *T. vulgaris* and IgA antibodies against *A. fumigatus*. In follow up studies of farmer's lung patients to determine the phase of the disease, antibodies of other Ig classes and against other microbes should also be measured.

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