

Comparison of *Aspergillus fumigatus* and *Aspergillus umbrosus* Antigens in Serological Tests of Farmer's Lung

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A comparison was made between *Aspergillus fumigatus* and *A. umbrosus* antigens regarding their usefulness in serological tests of farmer's lung. Mycelial and culture fluid antigens of both microbes were used to test sera of 30 Finnish farmer's lung patients for antibodies in the ELISA and in the gel precipitation (GP) test. Antibodies to *A. umbrosus* were more common than to *A. fumigatus*; this was especially evident in ELISA. ELISA gave more positive results with all four antigens than GP. Mycelial antigens showed more positive reactions in ELISA than the corresponding culture fluid antigens. The situation was reversed in GP. Selection of antigens in the diagnostic panel of farmer's lung requires careful consideration. Our results suggest that other *Aspergilli* (in the Finnish population *A. umbrosus*) might be preferable to *A. fumigatus* in the antigen panel.

Key words: *Aspergillus*; ELISA; farmer's lung; gel precipitation.

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Development of farmer's lung (FL) is associated with sensitization to microbial antigens. The presence of antibodies to microbes in the patients' sera indicates an exposure to the offending antigens and thus gives support to the diagnosis. The most commonly incriminated microbes are *Micropolyspora faeni*, *Thermoactinomyces vulgaris* and *Aspergillus fumigatus* (3, 8, 10, 12). The microbial flora of Finnish moldy hay seems to differ from that found elsewhere (10); it contains more fungi and less actinomycetes. Another characteristic of the hay is the large amount of fungi of the *Aspergillus glaucus*-group, especially *A. umbrosus*. Antibodies against both *A. fumigatus* and *A. umbrosus* are common in Finnish FL patients (10). Since fungi of the genus *Aspergillus* contain several cross-reacting antigens (4, 5) we compared in the present study the usefulness of antigens prepared from the two *Aspergillus* species, *A.*

fumigatus and *A. umbrosus* in the serological tests of farmer's lung.

MATERIAL AND METHODS

Study groups

Sera from 30 consecutive new cases of FL investigated at the Dept. of Pulmonary Diseases, Kuopio University Central Hospital in 1976-1977 were examined. All patients had been exposed to moldy hay or straw and had physical, radiological and physiological findings compatible with FL (1). The sera were kept frozen at -20° until examined.

Antigens

Antigens were prepared from *Aspergillus fumigatus* (Mycological Reference Laboratory,

London) and *Aspergillus umbrosus* 2236 isolated from Finnish moldy hay (10). *A. fumigatus* was grown in the synthetic medium VII (9) for 3–4 days at 37°C. *A. umbrosus* was cultivated in HSM-medium (2) supplemented with 10% NaCl at room temperature for 3–4 weeks.

The microbial cultures were centrifuged at $1000 \times g$ for 15 min. The extracellular material in the supernatant was concentrated by forced dialysis against polyvinylpyrrolidone K25 (50 $\times$) followed by dialysis against 0.05 M phosphate buffered saline pH 7.0 (PBS). The concentrate was used as the culture fluid antigen.

The mycelium was washed twice and the pellet suspended in an equal volume of PBS. The cells were disrupted mechanically (Ultra Turrax, Janke and Kunkel, Staufen i Breisgau, FRG) and by a 3-min ultrasonic treatment (MSE 1174 MK 2, Crawley, U.K.). After centrifugation at $1000 \times g$ for 15 min the supernatant was used as the mycelial antigen.

All antigens used in ELISA had protein concentrations of 50–100 $\mu\text{g/ml}$ and in gel precipitation 1–2 mg/ml.

Gel precipitation (GP)

A microtemplate modification of the immunoprecipitation technique (11) was used. The gel was 1% agarose in 0.1 M citrate- 0.2 M phosphate buffer (McIlvaine), pH 7.0. After incubation for 3 days the precipitates were washed and stained with Amido black.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed on microtiter plates as described previously (6, 7). The conjugate was horseradish peroxidase conjugated antihuman IgG (Orion Diagnostica, Finland). The substrate solution was composed of *O*-phenyldiamine, 3 mg/ml (Koch-Light Lab., Ltd.) and urea hydrogen peroxide (BDH Chemicals Ltd.) corresponding to 0.02% of H_2O_2 in 0.1 M citrate-phosphate buffer pH 5.5.

The absorbances at 492 nm were measured with a spectrophotometer (Shimadzu Seisakusho Ltd., Japan). Three dilutions of each serum were tested in ELISA. The absorbance curves

were drawn on a logarithmic scale of the serum dilutions.

The titer of a serum was expressed as the negative logarithm of the serum dilution which gave an absorbance of 0.5. The mean titer of the negative reference serum was calculated from five tests on all antigens. The mean titers $\pm$ standard deviations were 3.6 ± 0.19 for *A. fumigatus*, 4.0 ± 0.19 for *A. umbrosus* mycelial antigens and 3.2 ± 0.25 and 4.0 ± 0.25 for the corresponding culture fluid antigens. Serum titers exceeding these means by 2 SD were considered positive.

RESULTS

Gel precipitation

The reactivity of the sera in GP against *A. fumigatus* and *A. umbrosus* mycelial and culture fluid antigens is shown in Table 1. *A. umbrosus* antigens detected more positive reactions than *A. fumigatus* antigens. Ten sera were positive with the *A. umbrosus* mycelial antigen and 11 sera with the culture fluid antigen. The corresponding results for *A. fumigatus* antigens were 6 and 9, respectively. The difference between *A. umbrosus* and *A. fumigatus* was not, however, statistically significant ($P > 0.05$; McNemar's test).

The two sera which were positive with the mycelial antigens of both *A. umbrosus* and *A. fumigatus* showed a precipitation line with a reaction of identity. One of the sera showed only the precipitation line which was identical

Table 1
Number of sera with antibodies to *A. fumigatus* and *A. umbrosus* detected by gel precipitation

	Mycelial antigens <i>A. umbrosus</i>			Culture fluid antigens <i>A. umbrosus</i>		
	+	–	Total	+	–	Total
<i>A. fumigatus</i>						
+	2	4	6	8	1	9
–	8	16	24	3	18	21
Total	10	20	30	11	19	30

Table 2

Number of sera with antibodies to *A. fumigatus* and *A. umbrosus* detected by ELISA

<i>A. fumigatus</i>	Mycelial antigens <i>A. umbrosus</i>			Culture fluid antigens <i>A. umbrosus</i>		
	+	-	Total	+	-	Total
+	17	0	17	11	1	12
-	5	8	13	7	11	18
Total	22	8	30	18	12	30

with both antigens, the other one gave additional precipitation lines with *A. umbrosus*.

Eight sera were positive for the culture fluid antigens of both microbes. All precipitation lines of three of the sera showed reactions of identity. In addition, three of the sera showed specific precipitation lines, and two of the sera showed no lines of identity.

Enzyme-linked immunosorbent assay

The corresponding comparison by ELISA is presented in Table 2. Also in ELISA, *A. umbrosus* antigens gave more positive reactions than *A. fumigatus*. *A. umbrosus* mycelial antigen showed 22 positive reactions, 17 of which were also positive with *A. fumigatus*. No sera positive with *A. fumigatus* only were found. The culture fluid antigens gave fewer positive reactions than the mycelial antigens. The difference between *A. umbrosus* and *A. fumigatus* is almost significant

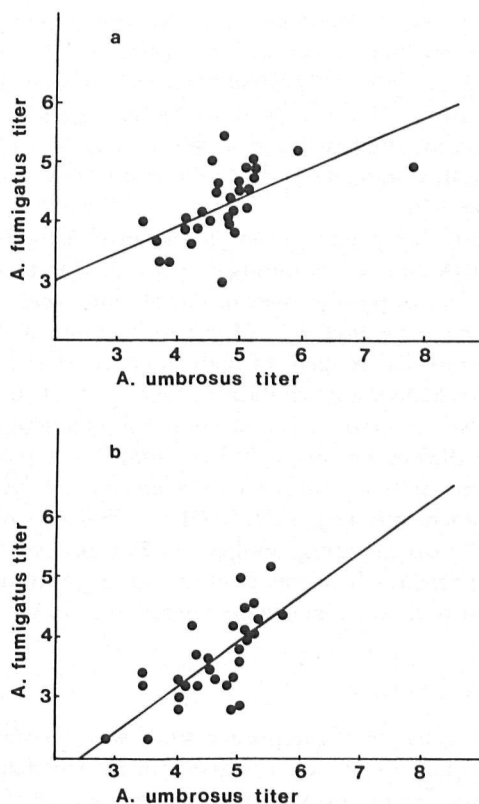


Fig. 1. Relationship between ELISA titers against *A. fumigatus* and *A. umbrosus* antigens. (a) Mycelial antigens, (b) Culture fluid antigens.

($0.05 > P > 0.01$; McNemar's test) both for mycelial and culture fluid antigens.

The reactivity of individual sera against *A. fumigatus* and *A. umbrosus* antigens in ELISA was

Table 3

Number of sera reacting with *A. fumigatus* and *A. umbrosus* mycelial and culture fluid antigens in ELISA and in gel precipitation (GP)

ELISA	Mycelial antigens						Culture fluid antigens					
	<i>A. fumigatus</i>			<i>A. umbrosus</i>			<i>A. fumigatus</i>			<i>A. umbrosus</i>		
	GP+	GP-	Total	GP+	GP-	Total	GP+	GP-	Total	GP+	GP-	Total
+	6	11	17	10	12	22	8	4	12	11	7	18
-	0	13	13	0	8	8	1	17	18	0	12	12
Total	6	24	30	10	20	30	9	21	30	11	19	30

compared by the simple regression ($y = a + bx$). The regressions between the titers against *A. fumigatus* and *A. umbrosus* mycelial as well as culture fluid antigens were highly significant (mycelial antigens $t_{28} = 4.286$; $P < 0.001$, culture fluid antigens $t_{28} = 5.245$; $P < 0.001$) (Fig. 1a and 1b).

The comparison between the two methods, ELISA and GP, is shown in Table 3. The total number of positive reactions by all four types of antigens was higher in ELISA than in GP. With the mycelial antigens of both fungi the sensitivity of ELISA was significantly higher ($P < 0.001$; McNemar's test). With the culture fluid antigens the difference was significant only for *A. umbrosus* ($0.01 > P > 0.001$; McNemar's test). All sera which were positive in GP also reacted with the corresponding antigen in ELISA, except one serum which was positive with *A. fumigatus* culture fluid antigen in GP but not in ELISA.

DISCUSSION

The purpose of the present study was to evaluate antigens of two *Aspergillus* species for diagnostic use by two serological techniques. Of the two techniques, ELISA gave more positive reactions than gel precipitation, indicating higher sensitivity of the method as described earlier (6, 7). All sera except one which were positive in gel precipitation were also found positive by ELISA.

In gel precipitation there were sera which were positive to both *A. fumigatus* and *A. umbrosus* antigens. Both reactions of identity and nonidentity were seen between the antigens of the two microbes. Therefore both or either one of the microbes were responsible for the appearance of the antibodies. Positive reactions with only one of the two *Aspergilli* suggested sensitization to this particular species. A majority of these reactions were against *A. umbrosus*. Sensitization to *A. umbrosus*, or to a closely related *Aspergillus* species, seemed to be more common in this group of FL patients than sensitization to *A. fumigatus*.

The antigen composition of *A. fumigatus* is dependent on the growth conditions (4, 5). Because of the different growth requirements

of *A. fumigatus* and *A. umbrosus* an antigenic comparison of the two *Aspergilli* grown under identical conditions did not seem feasible.

In ELISA *A. umbrosus* antigens also detected more positive sera than *A. fumigatus* antigens. The protein concentrations of the antigens of the two fungi were identical but there may have been differences in glycoprotein concentrations. A higher concentration of glycoprotein antigens in *A. umbrosus* preparations might result in a higher number of positive reactions. To some extent the better results obtained with *A. umbrosus* may be due to the high nonspecific binding of IgG on *A. fumigatus* mycelial antigen. A parallel study (to be published) showed that the reactivity pattern of farmer's lung patients and symptom-free controls against *A. umbrosus* was similar to that against *A. fumigatus* (7).

A previous serological analysis of 54 Finnish FL patients (10) showed that an antigen panel consisting of strains of *T. vulgaris*, *M. faeni* and *A. umbrosus* would be ideal to detect precipitin-positive patients. The results of the present study support the usefulness of *A. umbrosus* antigens in gel precipitation tests. We have previously used *A. fumigatus* antigen in ELISA and found a high rate of positive reactions with sera of FL patients (6, 7). The present results show that also in ELISA *A. umbrosus* antigen might be preferable to *A. fumigatus* for detecting sensitization in FL patients. If, however, sensitization to *Aspergilli* is of primary importance, then it would be advisable to include in the test panel both *A. fumigatus* and *A. umbrosus* and perhaps even other *Aspergillus* species.

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