

The allergenic and antigenic properties of spore extracts of *Aspergillus fumigatus*: A comparative study of spore extracts with mycelium and culture filtrate extracts

Henk F. Kauffman, Ph.D.,* Sicco van der Heide, M.Sc.,*
Frits Beaumont, M.D.,** Jan G. R. de Monchy, M.D.,*, and
Klaas de Vries, M.D.* Groningen, The Netherlands

The antigenic properties of conidial preparations of Aspergillus fumigatus were compared with mycelial and culture filtrate extracts by use of sera of patients with aspergillosis and hyperimmune rabbit serum. Biologic activities were tested by intracutaneous tests. The immunologic parameters used were: precipitating properties in double-diffusion, IgG binding by ELISA, IgE binding by enzyme inhibition assay, and by enzyme allergosorbent test. It has been demonstrated that the components that are released after disintegration of spores have positive titers in all immunologic assays. The immunologic properties of these spore plasma extracts are comparable with mycelial and/or culture filtrate extracts that are in accordance with the corresponding skin reactivities. Components that are released spontaneously from spores are only weakly positive or negative in the immunologic assays and demonstrate a very low biologic activity. (J ALLERGY CLIN IMMUNOL 73:567, 1984.)

In the clinical and immunologic studies of aspergillosis, two types of antigenic extracts of *A. fumigatus* have been used mainly for routine purposes, mycelial antigens, and CF concentrates. Antigens from both sources demonstrate IgG- and IgE-binding properties in a variety of lung diseases caused by *A. fumigatus*.¹ However, less information is present on the role of conidial antigens in disorders such as ABPA and AA. A possible role for *Aspergillus* S antigens in these disorders is supported by the observations of Stevens et al.,² demonstrating obstructive reactions in patients with allergic aspergillosis and aspergilloma after inhalation of *Aspergillus* antigen from disrupted Ss. Studies of the chemical composition of conidial and MY extracts of *A. fumigatus* demonstrated large differences in polysaccharides.³ In accordance with the

Abbreviations used

CF:	Culture filtrate
R:	Hyperimmune rabbit serum
EIA:	Enzyme inhibition assay
EAST:	Enzyme allergosorbent test
S:	Spore
SP:	Spore plasma
ABPA:	Allergic bronchopulmonary aspergillosis
AA:	Aspergillus asthma
MY:	Mycelium
SS:	Spore surface
DD:	Double-immunodiffusion

chemical differences, extracts of disrupted conidia were demonstrated to contain specific antigens (by use of R) that are not shared with mycelial and/or medium derived antigens.⁴ However, information on IgG- or IgE-binding properties of conidial antigens with sera of patients with aspergillosis is lacking.

The purpose of this study therefore was to investigate the antigenic and allergenic properties of extracts of Ss of *A. fumigatus* together with the antigenic extracts derived from MY and CF with sera of patients with aspergillosis.

From the Departments of Allergology* and Pulmonology,** Clinic for Internal Medicine, State University Hospital, Groningen, The Netherlands.

Received for publication Jan 27, 1983.

Accepted for publication Nov. 8, 1983.

Reprint requests: Henk F. Kauffman, Ph.D., Department of Allergology, Clinic for Internal Medicine, State University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

Since patients with ABPA or AA may demonstrate a rapid onset of symptoms after exposure to air contaminated with *Aspergillus*, the antigens that are spontaneously released from Ss of *A. fumigatus* were also studied in addition to the antigens from the S cell content after destruction of the Ss.

MATERIAL AND METHODS

Antigens

Three different sources for extracts of *A. fumigatus* have been used in this study: (1) CF antigens were prepared as described earlier⁵ from a strain isolated from a patient with aspergilloma. Generally late-phase cultures (incubation time ≥ 5 wk, see legend to Figs.) were used, but in some studies extracts of younger cultures (incubation time 10 days) were also used. (2) Antigens of mycelial origin were prepared from the same cultures as used for the CF-antigen preparation. The cultures used were grown for either 10 or 35 days of cultivation in a Sabouraud-glucose medium⁵ as indicated. MY destruction and preparation of the MY extract was done according to the procedure of Kim and Chaparas.⁶ (3) Ss of *A. fumigatus* (cultured and kindly supplied by Diephuis Laboratories) were obtained from cultures of the same strain on Sabouraud-glucose agar plates. Ss were harvested after 7 days of growth. Microscopic examination demonstrated less than 1% mycelial fragments.

S extracts were prepared by shaking 5 g of Ss in 250 ml of phosphate-buffered saline (pH 7.2). An amount of 0.1% of azide (NaN_3) was added in order to prevent both microbial growth and germination of the Ss. The suspension was agitated at 100 rpm (R.T.) for 48 hr followed by Seitz filtration. The supernatant was centrifuged for 30'/24,000 $\times$ g at 5° C, dialyzed against deionized water, and lyophilized. For dialyzing, Visking dialyze tubing (Visking Corp., Chicago, Ill.) was used with a molecular weight cut off of $\pm 10,000$ daltons. It is assumed that the components extracted in this way are mainly liberated from the spore surface (SS antigen). The (intact) Ss, collected after Seitz filtration, were homogenized in a small volume of water and destructed by Braun homogenization by use of glass beads with a diameter of 0.06 mm. Microscopic examination demonstrated about 60% destruction of the Ss. Glass beads and cell debris were removed by differential centrifugation (5'/300 $\times$ g and 30'/24,000 $\times$ g, respectively) and the supernatant, SP antigen, dialyzed and lyophilized. It was observed with both extracts (SS and SP) that some of the material was not redissolved (caused by autodenaturation) after lyophilization. Therefore in a separate experiment, the supernatants from SS and SP obtained after centrifugation were divided into two equal portions: one was treated as described above, and the other portion was concentrated by Amicon diaflow filtration (Amicon B.V., Oosterhout, The Netherlands) by use of UM-10 filters. The final volume of this second portion was made equal to the volume used for the corresponding lyophilized material.

The Amicon concentration procedure appeared to be milder, showing less autodenaturation. (Extracts demon-

strating denaturation were centrifuged before use in the different assays.) Extraction procedures using 5 g of intact Ss demonstrated yields of 84.8 mg of SS antigen (1.6%) and 219.6 mg of SP antigen (4.4%).

Sera used in this study are from patients with ABPA, *Aspergillus* asthma, and aspergilloma. The criteria used for diagnosis were (a) ABPA: history of obstructive reactions, infiltrations and (central) bronchiectasis on x-ray film of the chest, immediate skin reactivity to *A. fumigatus*, positive RAST to *A. fumigatus* antigen and elevated serum-total IgE, the presence of precipitating antibody to *A. fumigatus*, and peripheral blood eosinophilia; (b) *Aspergillus* asthma: history of obstructive reactions, normal chest radiograph, positive skin test and RAST to *A. fumigatus*, and absence of precipitating antibody to the antigen; (c) Aspergilloma: clinical and röntgenologic indications, e.g., hemoptysis and typical "fungus ball" with air crescent and the presence of precipitating antibody to *A. fumigatus*. Sera were routinely characterized for the presence of precipitating antibodies as described earlier,⁵ and specific IgE antibodies were measured by RAST (Pharmacia, Woerden, The Netherlands). Furthermore, serum was used from a pool of three rabbit sera (R) that was hyperimmunized with a CF antigen as described previously.⁵

Antigenic extracts were studied by means of (1) DD according to Ouchterlony by use of sera of patients with ABPA and aspergilloma and R; (2) an ELISA procedure measuring IgG-antibody titers as described earlier⁷; in short, 250 μ l of ELISA buffer and 1 μ l of patient serum were added in duplicate to wells of microtiter plates previously coated with *aspergillus* antigen. Human IgG antibodies bound to the antigen are detected by a double antibody assay by use of rabbit antihuman IgG followed by incubation with the enzyme conjugate (goat anti-rabbit alkaline phosphatase). Detection of the bound enzyme is performed by addition of substrate and spectrophotometrical determination of product formation at 405 nm; (3) an indirect method EIA for measuring IgE-binding properties; this was performed by use of the Phadezym RAST assay (Pharmacia) and *Aspergillus*-coated discs of Pharmacia. Samples of a pool of 10 sera from patients with elevated IgE antibodies against *A. fumigatus* (diluted 1:2 in the PDT buffer supplied with the Phadezym RAST assay) were incubated for 3 hr at 20° C with increasing concentrations of the antigenic extracts. Thereafter residual IgE was measured by Phadezym RAST assay. In this Phadezym RAST assay, the De_2 -anti-IgE serum is labelled with the enzyme beta galactosidase. Detection of the IgE-bound enzyme conjugate is done by the splitting of the enzyme from its carrier followed by the measurement of the liberated enzyme by addition of the substrate according to the producers instructions (Pharmacia). The reaction is stopped, after 2 hr of incubation at 37° C, by addition of 1.0 ml stop solution, and the absorbance is measured at 420 nm. The absorbance values obtained with the incubation buffer in the absence of serum and the values obtained with the pool serum of 10 healthy individuals (normal control serum) were used as controls; (4) a direct method EAST for measuring the IgE-binding

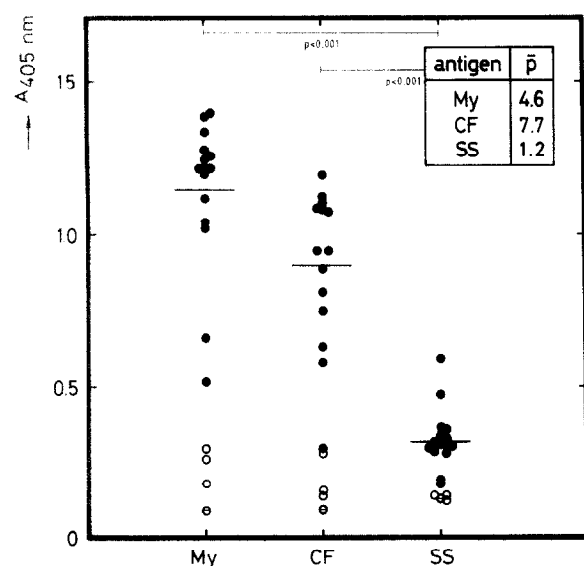


FIG. 1. IgG-ELISA antibody titers of individual sera and mean number of precipitates against extracts of *A. fumigatus*. ●, sera of patients with ABPA or aspergilloma ($n = 15$); ○, sera of healthy individuals; □, blank value in absence of sera; —, mean of IgG-ELISA titers of patient sera; MY extract, batch no. 053, 35 days of cultivation; CF, batch no. 053; SS antigen. *Inset* shows mean number of precipitates ($\bar{P}$) found by DD with patient sera. Level of statistical significance obtained by Student's paired t test is illustrated.

capacity of the extracts; Antigens (10 mg/ml) were immobilized on CNBr-activated paper discs as described by Karr et al.⁸ IgE binding of sera with elevated IgE antibodies against *A. fumigatus* was measured by use of the D_{E_2} -specific enzyme conjugate and the assay conditions of the Pharmacia Phadezym RAST assay as described under 3. All given titers of IgG and IgE measurements by RAST, EIA, and EAST are the means of duplicate measurements.

Assay of hemolytic complement consumption

The capacity of the different extracts to decrease the total hemolytic complement was measured by incubating 1 ml volumes of serum of two normal healthy individuals for 60 min at 37° C with 15 μ l (100 mg/ml) of the antigenic extracts. Total and residual complement hemolytic activity, before and after incubation of the extracts, was measured by CH_{50} titration according to the technique⁹ of Mayer and expressed as percent of the total hemolytic complement (100%).

Control experiments with 15 μ l of double-distilled water instead of antigenic extract did not reveal any decrease in hemolytic activity. Since the sera of the healthy volunteers did not contain antibodies against *A. fumigatus* as revealed by the absence of precipitating antibodies to the extracts, it is assumed that loss of complement was mainly the result of alternative pathway complement activation as reported

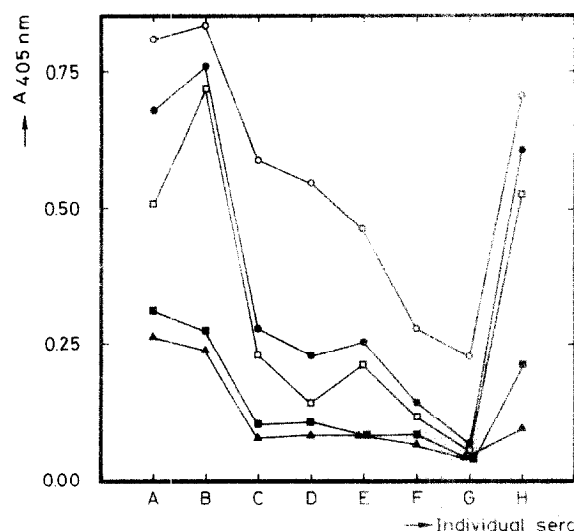


FIG. 2A. IgG-ELISA antibody titers of sera of patients with aspergilloma (A to D) and ABPA (E to H) against extract of *A. fumigatus*. Lines connect absorbance values obtained with single extract. ○—○, CF, batch no. 058; ●—● and □—□, SP extracts prepared by Amicon filtration and lyophilization, respectively (see Method and Materials). ■—■ and △—△, SS antigens Amicon filtrate and lyophilisate, respectively. All S extracts, batch no. 061

also by Marx and Flaherty.¹⁰ But the involvement of complement activation by the classic pathway cannot be excluded.¹¹

Intracutaneous tests were performed with the antigens dissolved in Ccca's solution (0.5 mg/ml) and filtered through Millex-GS filters (Millipore Nederland, Etten-Leur, The Netherlands). Skin tests were carried out on patients previously selected by demonstrating positive skin tests to the extracts used for routine diagnostic purposes. Skin titers were read 15 min after intracutaneous application of the antigens and scored as $\pm$, +, $++$, $+++$, and $++++$ corresponding with wheal diameters of 3 to 5, 5 to 7, 7.5 to 10, 10 to 12.5, 12.5 to 15, and >15 mm, respectively. For arithmetic analysis (mean reactivity) skin titers were expressed as 1 unit for $\pm$ and 2 units for each + score.

RESULTS

In Fig. 1 the ELISA IgG-antibody titers of an SS extract were compared with titers obtained with a CF and an MY extract by use of 15 sera of patients (aspergilloma and ABPA) and three control sera from healthy individuals. The mean number of precipitates of the different extracts against the patient sera is illustrated in the inset of Fig. 1. Both CF and MY antigens demonstrated elevated titers of IgG antibodies by ELISA and increased numbers of precipitates by DD.

In contrast to MY and CF antigens, the antigenic components spontaneously released by intact Ss (SS antigen) do demonstrate significantly lower ($p <$

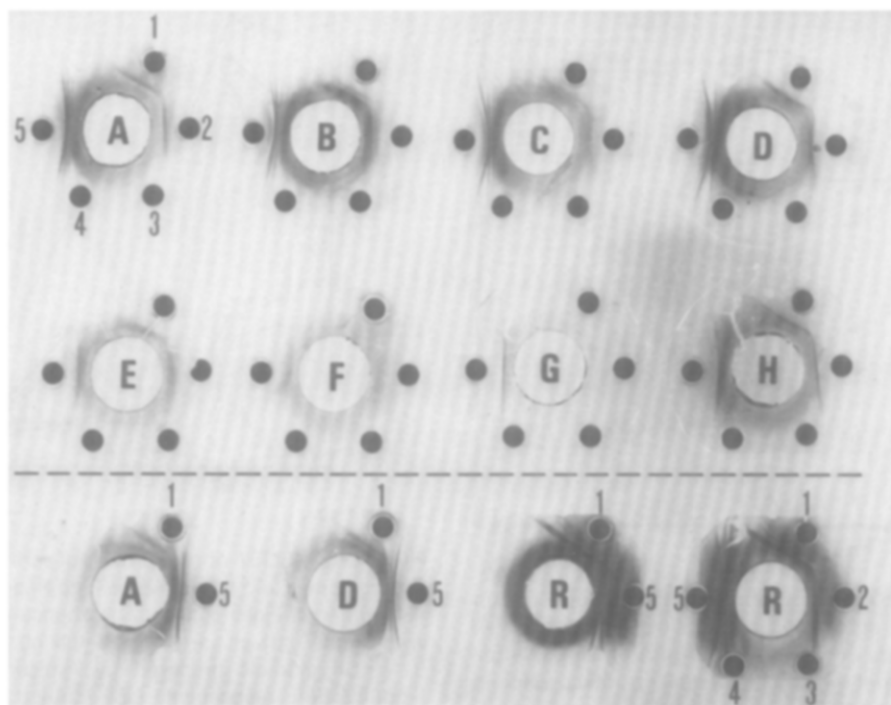


FIG. 2B. Precipitates against culture filtrate and S extracts of *A. fumigatus* formed by patient sera and R in DD. Sera A to H, patient sera as described under Fig. 2A. Antigenic extracts identical to extracts used in Fig. 2A; wells 1 and 2, SP extracts, Amicon concentrate and lyophilisate, respectively; wells 3 and 4, SS extracts, concentrate and lyophilisate; well 5, CF batch no. 058.

0.001) titers by ELISA, whereas in DD, only smaller numbers or absence of precipitates were observed. Fig. 2 summarizes a comparative study of SP-, SS-, and CF-antigenic extracts studied both by IgG-ELISA titers (Fig. 2, A) and DD (Fig. 2, B) by use of sera of patients with aspergilloma (sera A to D) and ABPA (sera E to H). In addition, R was also used in the DD. The CF antigen demonstrates more precipitating components and higher IgG-antibody binding by ELISA with all the sera of patients tested when compared with the SS and SP extracts. The SP extract demonstrates some (but fewer) precipitating components (6/8) and elevated IgG titers (7/8), whereas the SS antigen demonstrates much fewer precipitating components (1/8) and low or negative ELISA-IgG titers (3/8).

As with the observations on patient's sera, the R (lower part of Fig. 2, B, R, far right) also shows more precipitates against CF and SP antigen (wells 5 and 1, and 2, respectively) than against the SS antigen (well 4) which hardly showed any precipitates.

Cross-reactivity of SP and CF antigen (wells 1 and 5, respectively) as studied in the lower part of Fig. 2, B (sera A, D, and R) demonstrate a partial identity of the main precipitate found with SP by use of patient sera D. With R, noncrossreacting components can

also be observed. Although the lyophilized extracts did demonstrate more autodenaturation, the precipitation patterns between lyophilized and Amicon (Amicon B.V.) concentrated extracts (Fig. 2, B, wells 1, 3 and 2, 4, respectively) do not demonstrate differences, whereas ELISA titers are only slightly less compared with the concentrated extracts. This indicates that the immunologically important antigenic components are stable molecules and are only slightly influenced by the autodenaturation process.

The IgE binding properties of both SS and SP antigen were compared with CF and MY antigen by use of EIA (Fig. 3). With the MY extract a total inhibition of IgE binding was found, indicating total identity with allergens of the Pharmacia disc. With the CF antigen (CF, 38 days of culture) an inhibition of 70% was obtained at a similar antigen concentration, but at higher antigen concentrations no further inhibition was found, indicating partial identity with the antigen bound to the disc.

The SP antigen did demonstrate some inhibition at higher antigen concentration, whereas the SS antigen did not influence the IgE titers, indicating a limited and nonidentity with the immobilized antigen, respectively.

Besides this *indirect* IgE-binding assay, the an-

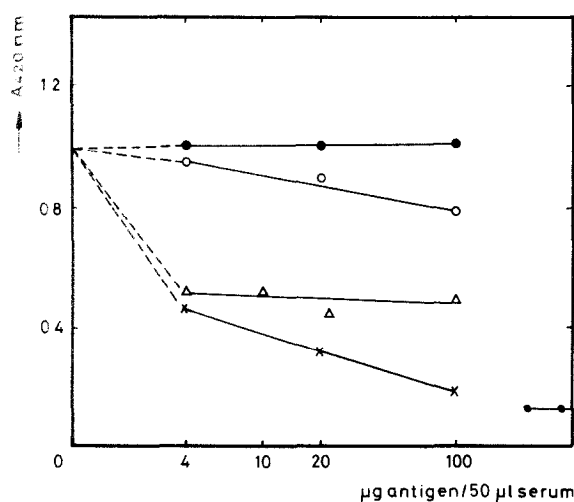


FIG. 3. IgE-binding properties of extracts of *A. fumigatus* by means of EIA. Extracts tested, SS, ●—●; SP, ○—○; CF of late-phase culture (38 days of cultivation), △—△; MY of 10-day culture, X—X. ●—●, on right side; ELISA-IgE titer found with pool of 10 normal control sera.

tigenic extracts were also immobilized on cellulose discs and tested in a direct IgE-binding assay EAST by use of sera with elevated IgE antibodies against *A. fumigatus*. As is demonstrated in Fig. 4, both MY, CF, and SP demonstrated high IgE-binding properties, whereas the titers with SS antigen were much lower. High titers of IgE antibodies against the SP antigen were found in those sera that also demonstrated high titers of IgE antibodies against the CF and MY antigens.

The biologic activities of the extracts MY, CF, and SP, as studied by their skin reactivity (Table I), are in agreement with the IgE-binding properties of these extracts (Fig. 4). In Table I the skin reactivities have been summarized for 18 patients with the use of CF, MY, SP, and SS extracts. Calculation of the mean reactivity (see Material and Methods) demonstrates high skin reactivity for both CF, MY, and SP antigen, whereas the SS is much less reactive. In addition similar reactivities were found for CF, MY, and SP antigen in individual patients (Table I).

Some patients with weak reactions (+) to the routine *Aspergillus* extract demonstrated negative reactions with all or almost all the extracts tested (Table I, patient no. 1, 2, 4, 8, 14, and 17). These negative reactions indicate that positive reactions are due to specific sensitization and not due to aspecific reactivity of the extracts.

In agreement with the serologic observation as described above, no differences are found between the SP antigens after lyophilization (SP 1) and Amicon concentration (SP 2). This indicates that the autodenaturation, caused by the lyophilization proce-

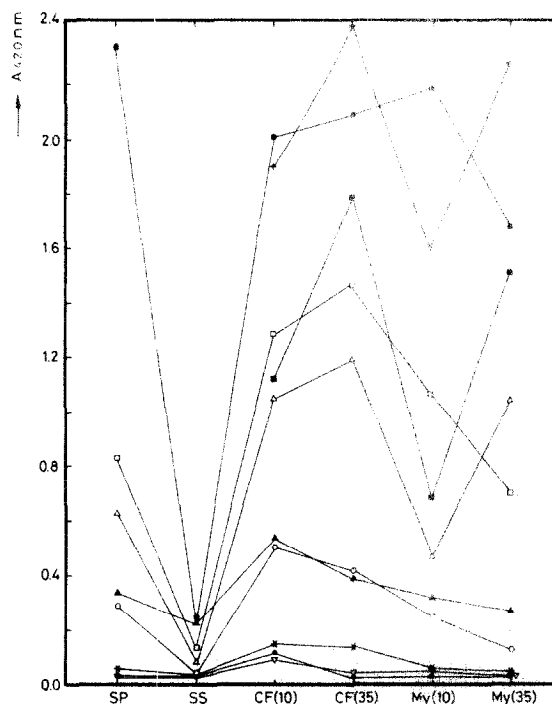


FIG. 4. IgE-binding properties of extracts of *A. fumigatus* by means of direct EAST. ●—● and ○—○, absorbance values found with incubation buffer (without serum) and normal pool serum, respectively. Other symbols represent absorbance values found with individual sera of patients with ABPA, both in acute phase and in different stages of remission. Extracts tested, SP, SS, CF(10), CF(35), MY(10), and MY(35). Numbers in parentheses represent days on cultivation. Lines connect absorbance values found with individual sera.

dures and observed in SP 1 has not influenced its biologic activity.

Complement activation

A. fumigatus antigens are known for their interaction with the complement system independent of the presence of IgG antibodies.¹⁰ This complement activation might possibly influence biologic activities in an aspecific (nonimmunologic) way. Therefore extracts of *A. fumigatus* (CF and MY extracts of 10-day and 35-day cultures, SP, and SS) were tested for their complement activating properties by use of sera of two healthy control individuals. In Fig. 5 the titers of the residual complement hemolytic activities measured by CH₅₀ titration are revealed after 1-hour incubation at 37° C (see Material and Methods). CF antigens of a young culture (CF 10 days of culture) and the SS antigen demonstrate less than 20% activation of the complement system, whereas CF antigen of a 35-day culture and the SP antigen demonstrate activations between 30% to 45%.

The MY extracts did reveal much higher comple-

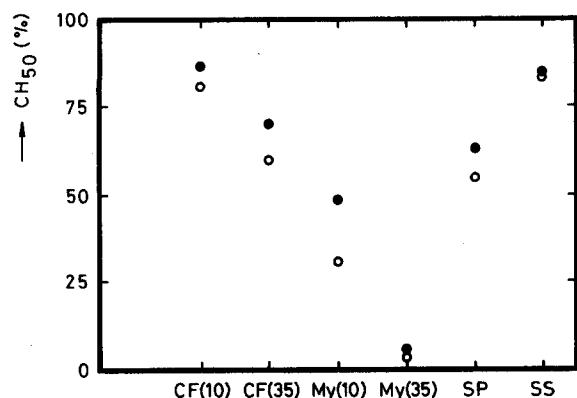


FIG. 5. Decrease in hemolytic activity of complement measured by CH_{50} titration in two normal sera after incubation with extracts of *A. fumigatus* expressed in percentages of the untreated sera. Extracts tested, see Fig. 4. CH_{50} titers demonstrated are means of duplicate experiments.

ment-activating properties demonstrating consumptions of 50% to 80% and 95% for 10-day and 35-day cultures, respectively. This suggests that the biologic activity of the MY extracts may possibly be influenced by their high complement inactivating properties.

DISCUSSION

In this comparative study of antigenic extracts of *A. fumigatus* from MY, CF, and S origin, it was found that the extract from the conidia after destruction (SP) demonstrated both IgG- and IgE-binding properties. This is in agreement with the biologic activity of SP as measured by skin tests (Table I) and after inhalation of S extract.² Although the number of precipitates and titers of ELISA-IgG antibodies against SP antigen are in general smaller when they are compared with the CF antigen, the biologic activities are similar. Similar to the findings of Scholer,⁴ a partial identity was found with the SP and CF antigens when these antigens were studied by DD with the use of R, whereas several conidial antigens were observed to be noncross-reacting with the CF antigens. Sera of patients with aspergilloma demonstrated one precipitating component with the SP antigen, partially cross-reacting with a CF precipitate (Fig. 2, sera A and D).

When the IgE-binding properties of the extracts were studied by means of EIA, both MY and CF demonstrated a marked inhibition of the IgE titers (by use of Pharmacia discs loaded with *A. fumigatus*).

Although the SP antigen did not demonstrate an inhibition in this EIA, an efficient binding of IgE antibodies was found in the direct IgE-binding assay EAST. This indicates that IgE-binding components, present in conidial plasma extracts, are not or only

TABLE I. Skin tests with different extracts of *Aspergillus fumigatus*

Patients No.	Type of extracts				
	CF	MY	SS	SP1	SP2
1	2	0	0	0	—
2	0	1	0	0	—
3	6	6	4	6	—
4	0	0	0	0	—
5	5	5	0	3	—
6	0	1	0	1	—
7	2	2	0	2	—
8	0	0	0	4	—
9	0	—	—	1	—
10	2	1	0	3	—
11	5	6	4	4	—
12	—	—	0	2	1
13	4	4	0	2	—
14	—	—	0	0	0
15	—	—	0	5	5
16	—	—	3	6	6
17	—	—	0	0	0
18	6	6	1	4	4
No. of tests	13	12	17	18	6
Mean	2.5	2.6	0.7	2.4	2.7
SD	2.4	2.5	1.4	2.1	2.7

Arithmetical analysis as described under Material and Methods;
— = not done.

slightly cross-reacting with extracts of either MY or CF origin.

In contrast to the conidial plasma antigen, the material that is released spontaneously from intact Ss (which is 26% of the total extractable material) did demonstrate low immunologic activity in DD, IgE-ELISA titers, and IgE-binding assays EAST and EIA. This low immunologic activity is also reflected in a very low biologic activity (Table I). These observations indicate that liberation of antigenic components after inhalation of Ss only will occur after destruction (or germination?) of the Ss. The lack of spontaneous liberation of antigenic (allergenic) components from the Ss of *A. fumigatus* are in sharp contrast with the rapid liberation of the allergenic components from pollen¹² and house dust mite allergen.¹³ Destruction of Ss by phagocytosis takes several hours,¹⁴ and the intracellular killing will prevent liberation of the antigenic components into the extracellular space. Also, germination of nonphagocytosed Ss will last several hours.¹⁵ Therefore the rapid onset of obstructive reactions in patients with ABPA and AA after inhalation of air contaminated with fungi (in stables and barns) is difficult to explain as being caused by the inhalation of intact Ss. It is, however, possible that inhaled de-

caying fungal material is an important source of easy releasable antigenic components.

Furthermore it must be noted that the extraction conditions are different from those in the lung. The influence of components as surfactant is unknown. Therefore the influence of surface-active components on the releasability of allergenic components from Ss of *A. fumigatus* needs further study.

Complement activation, studied in sera without detectable precipitating antibodies, was demonstrated to be limited for CF and S antigens. In contrast, the MY extract demonstrated a considerable complement consumption. This is in accordance with similar observations by use of endocellular extracts of *A. fumigatus*.^{10, 11} This activity was demonstrated to be caused by both activation of the classic pathway¹¹ and alternative pathway of complement.^{10, 11} In addition, it is interesting to note that, similar to the observations with *Micropolyspore faeni* and *Thermoactinomyces vulgaris*,¹⁰ the most complement-activating components were found in the endocellular extracts (MY) and, to a lesser extent, in the other antigenic preparations. However, the degree of complement activation involved in skin reactivity with the MY extract appears to be of minor importance since the reactivity of the MY extract is similar to that of the CF or SP extracts, and also negative reactions were observed with the MY antigen (Table I) excluding aspecific effects.

We would like to thank Mr. H. Lange and Mrs. M. Nakken for their skillfull technical assistance, Mrs. J. Kruitbosch-Koopmans for her expert secretarial help, and Mrs. B. Schilizzi for critical reading and linguistic correction of the manuscript.

REFERENCES

1. Bardana EJ: The clinical spectrum of aspergillosis. Part 1. Epidemiology, pathogenicity, infection in animals and immunology of *Aspergillus*. CRC Crit Rev Clin Lab Sci 13:21, 1981
2. Stevens EAM, Hilvering S, Orie NGM: Inhalation experiments with extracts of *Aspergillus fumigatus* on patients with allergic aspergillosis and aspergilloma. Thorax 25:11, 1970
3. Barreto-Bergter E, Gorin PA, Travassos LR: Cell constituents of mycelia and conidia of *Aspergillus fumigatus*. Carbohydr Res 95:205, 1981
4. Scholer HJ: Specific mycelial and conidial antigens from *Aspergillus fumigatus*. Haller Rde, Suter F, editors. In Aspergillosis and farmer's lung in man and animal. Bern, 1974, Hans Huber, p 45
5. Kauffman HF, Vries K de: Antibodies against *Aspergillus fumigatus*. I. Standardization of antigenic composition. Int Arch Allergy Appl Immunol 62:252, 1980
6. Kim SJ, Chaparas SP: Characterization of antigens from *Aspergillus fumigatus*. Am Rev Respir Dis 118:547, 1978
7. Kauffman HF, Beaumont F, Meurs H, van der Heide S, de Vries K: Comparison of antibody measurements against *Aspergillus fumigatus* by means of double-diffusion and enzyme-linked immunosorbent assay (ELISA). J ALLERGY CLIN IMMUNOL 72:255, 1983
8. Karr PM, Wilson MR, Anicetti VR, Lehrer SB, Butcher BT, Salvaggio JE: An approach to fungal antigen relationship by radio allergosorbent test inhibition. J ALLERGY CLIN IMMUNOL 67:194, 1981
9. Mayer MM: Complement and complement fixation. In Kabat EA, Mayer MM, editors: Experimental immunochemistry, ed. 2. Springfield, Ill., 1961, Charles C Thomas, p 133
10. Marx JJ, Flaherty DK: Activation of the complement sequence by extracts of bacteria and fungi associated with hypersensitivity pneumonitis. J ALLERGY CLIN IMMUNOL 57:328, 1976
11. De Bracco MM, Budzko DB, Negroni R: Mechanisms of activation of complement by extracts of *Aspergillus fumigatus*. Clin Immunol Immunopathol 5:333, 1976
12. Marsh DG, Benin L, Bruce CA, Lichtenstein LM, Hussain R: Rapidly released allergens from short ragweed pollen. I. Kinetics of release of known allergens in relation to biological activity. J ALLERGY CLIN IMMUNOL 67:206, 1981
13. Tovey ER, Chapman MD, Platts-Mills TAE: Mite faeces are a major source of house dust allergens. Nature 289:592, 1981
14. Schaffner A, Douglas H, Brande A: Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*. J Clin Invest 69:617, 1982
15. Hawker LE: Germination: morphological and anatomical changes in the fungus spore. In Maedelin MF, editor: London, 1966, Butterworth & Co, p 151