

# Partial characterization of a rapidly released antigenic/allergenic component (Ag 5) of *Aspergillus fumigatus*

M. Louise Taylor, BSc,\* and Joan L. Longbottom, PhD London, England

*An antigenic component of Aspergillus fumigatus, which was responsible for inducing an initial early antibody response in rabbits immunized with A. fumigatus germings, has been identified and partially characterized. By immunofluorescent antibody and ELISA techniques, this antigen was demonstrated to be present on the germing surface, although it was also detected in supernatants of conidial germings within 1 hour and was present in all shake and stationary culture-filtrate extracts. By incorporation of a monospecific antiserum to this component in an intermediate gel of the reference self-crossed radioimmuno-electrophoresis pattern for allergic bronchopulmonary aspergillosis sera, it was recognized as Ag 5 and was also demonstrated to bind specific IgE. Further immunochemical analyses have revealed that Ag 5 is relatively heat labile, does not bind to concanavalin A, and has a molecular weight of approximately 35 kd. This rapidly released antigenic/allergenic component may play an important role in the initiation of immunologic responses in patients with allergic bronchopulmonary aspergillosis. (J ALLERGY CLIN IMMUNOL 1988;81:548-57.)*

*Aspergillus fumigatus* is known to produce many antigenic and allergenic components stimulating IgG and IgE antibodies, the detection of which is used to aid the diagnosis for patients with various disorders associated with the fungus. Most of the extracts used have been prepared either from disrupted mycelium of short-term (3- to 4-day) cultures or from long-term (4- to 6-week) culture filtrates of the fungus grown on a fully synthetic medium. Since the latter are prepared during the autolytic phase of growth, they are believed to contain not only extracellular (metabolic) components but also all intracellular (cytoplasmic) components as a result of lysis and, hence, consist of a full spectrum of antigens and allergens.

Although such preparations are necessary for diagnostic purposes, there is little information with regard to release or recognition of antigen/allergen from conidia (spores) or germinating conidia, which may be important in the initial stimulation and subsequent triggering of an immunologic response. Extracts of

## Abbreviations used

|        |                                         |
|--------|-----------------------------------------|
| ABPA:  | Allergic bronchopulmonary aspergillosis |
| Con A: | Concanavalin A                          |
| RIE:   | Rocket immunoelectrophoresis            |
| FRIE:  | Fused-rocket immunoelectrophoresis      |
| PBS:   | Phosphate-buffered saline               |
| XIE:   | Crossed immunoelectrophoresis           |
| XRIE:  | Crossed radioimmuno-electrophoresis     |
| HBSS:  | Hanks' balanced salt solution           |

disrupted conidia have been demonstrated to contain specific antigens not shared with mycelial or culture filtrate antigens,<sup>1</sup> although both their biologic and immunologic properties were found to be comparable.<sup>2</sup> By contrast, components spontaneously released from spores appeared to lack or contain only low levels of either activity.

During our studies of specific antigenic components in relation to IgG responses in patients with ABPA, two major antigens, defined as those to which most ABPA sera contained specific antibodies, have been characterized, namely, Ag 7<sup>3</sup> and Ag 13.<sup>4</sup> However, both these antigens have been regarded as intracellular in origin, since neither component appeared in the culture filtrate before the fungus was in its autolytic phase of growth.<sup>5</sup>

In the present study, an antigenic component inducing an initial early antibody response in rabbits

From the Department of Allergy and Clinical Immunology, Cardiothoracic Institute, Brompton Hospital, London, England.

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Reprint requests: Joan L. Longbottom, PhD, Department of Allergy and Clinical Immunology, Cardiothoracic Institute, Brompton Hospital, Fulham Road, London SW3 6HP, England.

\*Present address: Department of Anatomy and Embryology, University College, London, Gower St., London WC1E, England.

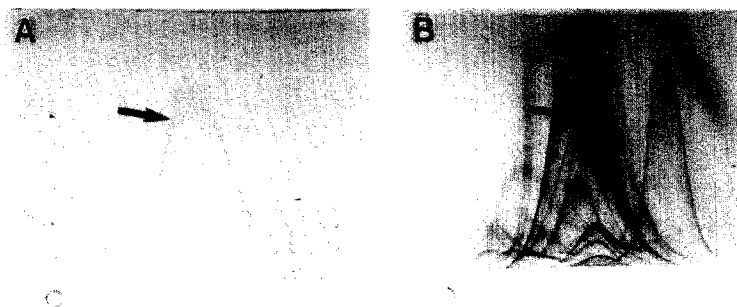


**FIG. 1.** XRIE tests with culture-filtrate antigen (90 µg) comparing the initial antigermling antisera in **A**, with the reference hyperimmune rabbit antiserum in **B**. Antisera incorporated at 7.5 µl/cm<sup>2</sup>. Recognized peak is indicated by arrow.

immunized with "germlings" (germinated conidia) has been identified as a rapidly released conidia/germling surface component, which also binds both specific IgG and IgE of ABPA sera. This component was further characterized immunochemically.

## MATERIAL AND METHODS

### *A. fumigatus* antigen preparations

**Germination of *A. fumigatus* conidia.** *A. fumigatus* (IMI No. 16030) conidia were harvested with PBS containing 0.1% Tween 20 from approximately 7-day-old surface cultures grown on Sabouraud's dextrose agar. These conidia were then incubated in a synthetic asparagine medium<sup>6</sup> at 30° C until approximately 70% of the conidia had germinated (15 hours) and further growth was inhibited by the addition of formaldehyde (final concentration 4%) to the culture medium. The resulting germinated conidia, "germlings," were washed with PBS before fixing onto microscope slides for use in the immunofluorescence assay or binding to microtiter plates for use in an ELISA system. The medium from these cultures was also dialyzed and freeze-dried.

**Long-term stationary cultures.** Batches of *A. fumigatus* were grown at 25° C for 4 to 5 weeks on the synthetic asparagine medium (as previously described).<sup>3,4</sup> The culture filtrate was separated from the mycelial mat, membrane filtered, dialyzed against frequent changes of distilled water, and freeze-dried. A reference preparation (B27), similarly prepared, was the same as used in previous studies.<sup>7</sup>

**Short-term shake cultures.** Cultures were incubated in the above medium with vigorous shaking at 35° C for periods of 1, 2, 3, 4, and 7 days, and after each time point, duplicate flasks were harvested. The culture filtrates were separated from the mycelium by coarse filtration, membrane filtered, dialyzed, and freeze-dried.

**Extraction of conidial surface antigens.** Approximately 200 mg of *A. fumigatus* conidia was incubated, with shaking, at 25° C in either 20 ml of Coca's solution or 20 ml of HBSS (Gibco, Paisley, Scotland). After periods of 1, 4, and 24 hours, conidia were removed by filtration through a membrane filter (0.45 µm) and reincubated with a further

20 ml of buffer. The extraction medium was dialyzed and freeze-dried, and the resultant antigen extracts were examined for the presence of Ag 5 with RIE.

### Human sera

Sera were obtained from patients with ABPA, and a standard serum pool was prepared from six sera with high IgE titers and precipitating IgG.<sup>7</sup> Serum was also obtained from two patients with an aspergilloma. (This developed in one patient subsequent to a diagnosis of ABPA.) All sera were stored at -20° C.

### Rabbit antisera

Antisera were raised in New Zealand white/Dutch lop crossbred rabbits.

**Germling/spore antisera.** Rabbits were immunized with approximately  $2 \times 10^8$  formalin-fixed conidia or 15-hour germlings that were washed and resuspended in 1 ml of saline, and emulsified in 3 ml of Freund's incomplete adjuvant and 25 mg of aluminum orthophosphate. Injections were administered intramuscularly into the thigh and intradermally in multiple sites into the back. This was repeated after 4 weeks, and rabbits were subsequently boosted every 4 to 6 weeks with germlings or spores in saline.

**Pooled reference antiserum.** Rabbits were immunized in the same regimen as for the germling antiserum with 10 mg of culture-filtrate extract (obtained from several batches) in 1 ml of saline emulsified with 3 ml of Freund's incomplete adjuvant and aluminum orthophosphate. High-titer antisera (as determined by XIE) from several bleeds from five rabbits were pooled for use as a reference hyperimmune antiserum.

**Monospecific antiserum.**<sup>8</sup> An Ag 5 monospecific antiserum was raised by excising the main precipitin peak from a series of eight XIE plates of culture filtrate against the initial germling antisera (Fig. 1, A). The excised antigen-containing agarose was dispersed in isotonic saline by sonication at 4° C, emulsified in Freund's incomplete adjuvant, and used to immunize rabbits (as above).

Monospecific antisera to two previously characterized major antigens, Ag 7<sup>3</sup> and Ag 13,<sup>4</sup> and antiserum (prepared as above) to *Alternaria alternata* antigenic extracts were also used for some tests.

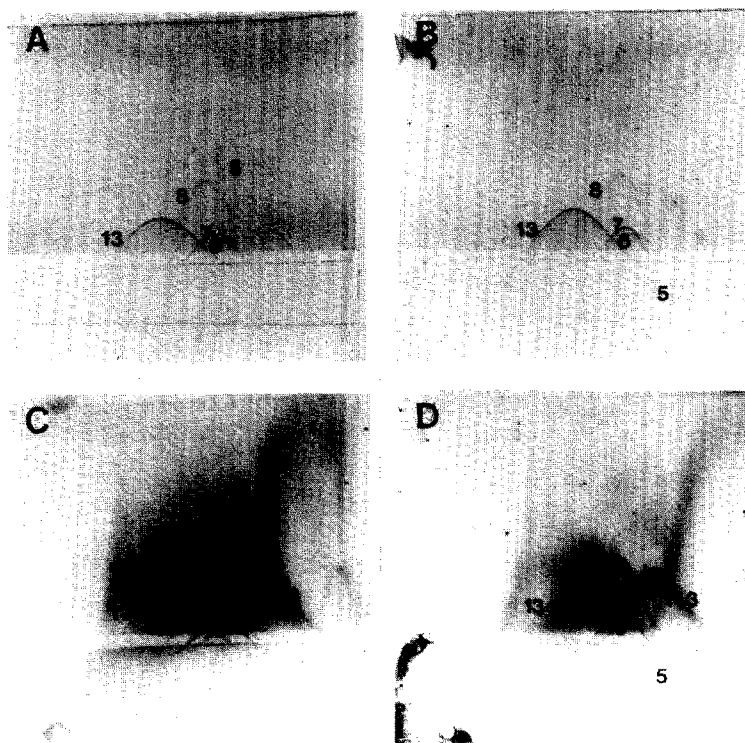


**FIG. 2.** Self-XRIE tests of the reference ABPA serum pool ( $25 \mu\text{l}/\text{cm}^2$  gel) with *A. fumigatus* antigen B27 ( $18 \mu\text{g}$ ). An intermediate gel incorporating Ag 5 monospecific antiserum ( $10 \mu\text{l}/\text{cm}^2$ ) was included in **B** together with its autoradiograph **D**. **A** and **C**, Corresponding control tests. Numbering of the main antigenic peaks is the same as in earlier studies.<sup>7, 11, 12</sup>

### Immunofluorescence assay

Preparations of antigen slides and the assay procedure were according to the method of Gordon et al.<sup>9</sup> Briefly, one drop of a germling suspension ( $2 \times 10^7/\text{ml}$ ) was air-dried on a glass microscope slide, fixed in an ethanol:water:chloroform (6:1:3) mixture for 4 minutes, followed by 95% ethanol for 4 minutes, and finally rinsed in water, dried, and stored at  $-20^\circ \text{C}$ . Serial twofold dilutions of test and control antisera were spread evenly over the antigen spot and incubated in a moist chamber at  $37^\circ \text{C}$  for 1 hour. Slides were washed in three changes of PBS, air-dried, and overlayed with one drop of goat antirabbit IgG fluorescein conjugate (Sigma Chemical Co., Poole, Dorset, England), and diluted 1:50 for 1 hour at  $37^\circ \text{C}$  in a moist chamber. Slides were mounted in 90% glycerol and 1% phenylene diamine (Sigma Chemical Co.), and fluorescence was read on a scale of 0 to 5 with anything greater than the value obtained with a PBS control (i.e., without serum), generally 2+, considered positive.

### Crossed and self-crossed immunoelectrophoresis and radioimmunoelectrophoresis

These tests were performed as previously described.<sup>7, 10, 11</sup> In some tests, an intermediate gel, containing a second antiserum, was incorporated between the antigen and the main antibody containing gel. Such XIE plates were compared with control plates without antiserum in the intermediate gel.

### Rocket immunoelectrophoresis

Gel plates were prepared as for XIE, and  $3 \mu\text{l}$  of each antigenic sample was loaded into wells 1 cm apart and electrophoresed directly at 1.5 V/cm overnight into gel containing Ag 5 monospecific antiserum.

### Fused-rocket immunoelectrophoresis

FRIE for monitoring the separation of antigenic components by gel filtration chromatography was as previously described<sup>12</sup>;  $3 \mu\text{l}$  of alternate column fractions, which were placed in zigzag rows of sample wells, was allowed to diffuse for 1 hour before electrophoresis (1.5 V/cm overnight) into gel containing rabbit *A. fumigatus* antiserum. An intermediate gel containing Ag 5 monospecific antiserum was incorporated in the FRIE profile to determine the fractions containing this component.

### Gel filtration chromatography

Gel filtration was carried out at room temperature on a 2.5 cm by 85 cm column of Sephacryl S-200 (Pharmacia, Milton Keynes, U.K.). The column was equilibrated in 0.05 mol/L of ammonium hydrogen carbonate ( $\text{NH}_4\text{HCO}_3$ ) buffer with a flow rate of 20 ml/hr, and the eluate, monitored at 280 nm (Uvicord II, LKB, Bromma, Sweden), was collected in 4-ml fractions. The column was calibrated by markers of known molecular weight. The sample, an ammonium sulfate-precipitated protein fraction of an *A. fumigatus* culture-filtrate extract (180 mg), was loaded

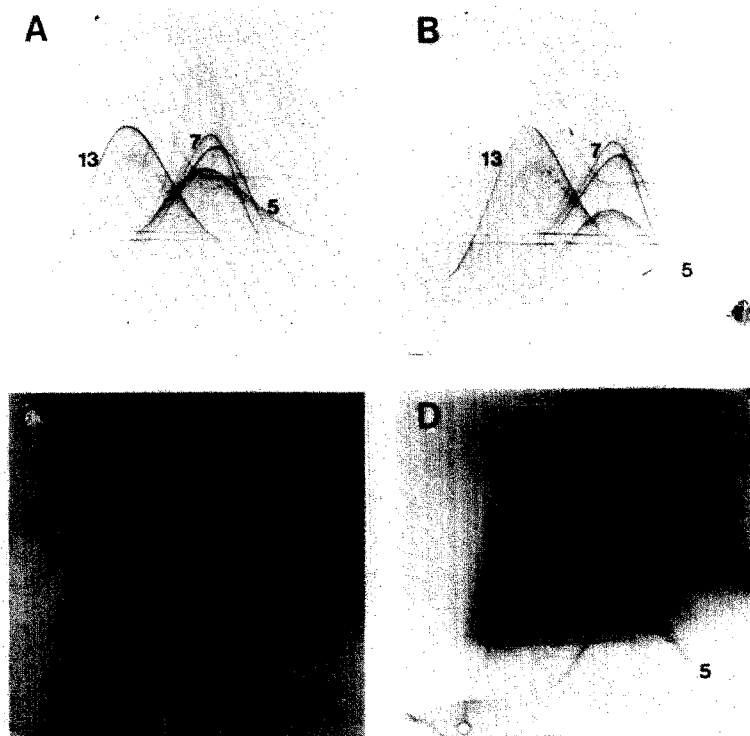


**FIG. 3.** Self-XRIE test of the serum of a patient with an aspergilloma and ABPA (12.5  $\mu\text{l}/\text{cm}^2$  gel) with *A. fumigatus* antigen B27 (90  $\mu\text{g}$ ). **A** and **C**, Control protein-stained plate and autoradiograph, respectively. **B-D**, Correspondingly, but incorporating Ag 5 monospecific antiserum in the intermediate gel (10  $\mu\text{l}/\text{cm}^2$ ). Note intensity of both protein stain and associated radioactive uptake on Ag 5 peak. Other major antigens, Ag 7 and Ag 13, as well as major allergen Ag 3, are indicated for this serum.

in 5 ml of buffer, and the elution profile was monitored by FRIE.

#### Absorption of specific IgG from monospecific antiserum

One milliliter of Ag 5 monospecific antiserum was incubated overnight with  $2 \times 10^7$  washed, formalin-fixed germlings in 50  $\mu\text{l}$  of PBS. Germlings were removed by centrifugation at 12,000 rpm, and the absorbed serum was compared with a control serum (1 ml of serum plus 50  $\mu\text{l}$  of PBS) in XIE tests.

#### ELISA with solid phase-bound germinated conidia

Germlings were bound to microtiter plates with Con A. Wells were coated with 200  $\mu\text{l}$  of Con A (Pharmacia; 100  $\mu\text{g}/\text{ml}$  PBS) and incubated for 1 hour at 25° C. The lectin solution was removed, the plates were washed with PBS, and then each well received approximately  $2 \times 10^8$  washed, formalin-fixed germlings in 100  $\mu\text{l}$  of PBS. After 1 hour at 25° C, protein-binding sites were blocked by incubation with 3% bovine serum albumin in PBS for an additional hour; 200  $\mu\text{l}$  of diluted (1:100) test and control antisera was applied per well, and the plate was incubated at 37° C for 1 hour. After washing three times with PBS/Tween (0.05%), 200  $\mu\text{l}$  of alkaline phosphatase-conjugated goat antirabbit

IgG (ICN, Stoke Poges, Berkshire, England) diluted (1:500) in PBS/Tween was added and incubated for 1 hour at 37° C. After further washings, freshly prepared substrate *P*-nitrophenol phosphate (Sigma Chemical Co., Poole, Dorset, England) at 1 mg/ml in 9.7% diethanolamine, pH 9.8, was added to the wells, and the reaction stopped by the addition of 50  $\mu\text{l}$  of NaOH. The optical density at 405 nm was determined with an automatic plate reader (Flow Laboratories, Rickmansworth, Hertfordshire, England), and results are expressed as optical density at 405 nm.

## RESULTS

#### Characterization of rabbit antisera

In XIE with a culture-filtrate antigen and a spore-surface extract, precipitating (IgG) antibodies were not detectable in the sera of rabbits after immunization with formalin-fixed *A. fumigatus* conidia. Antisera from three rabbits immunized with germlings initially recognized one main antigenic component of the culture-filtrate extract (Fig. 1, A), although with repeated immunization, several more precipitin peaks became visible (not presented). Fig. 1, B, illustrates the number of antigenic components of this culture-filtrate extract observed by the pooled hyperimmune reference antiserum.

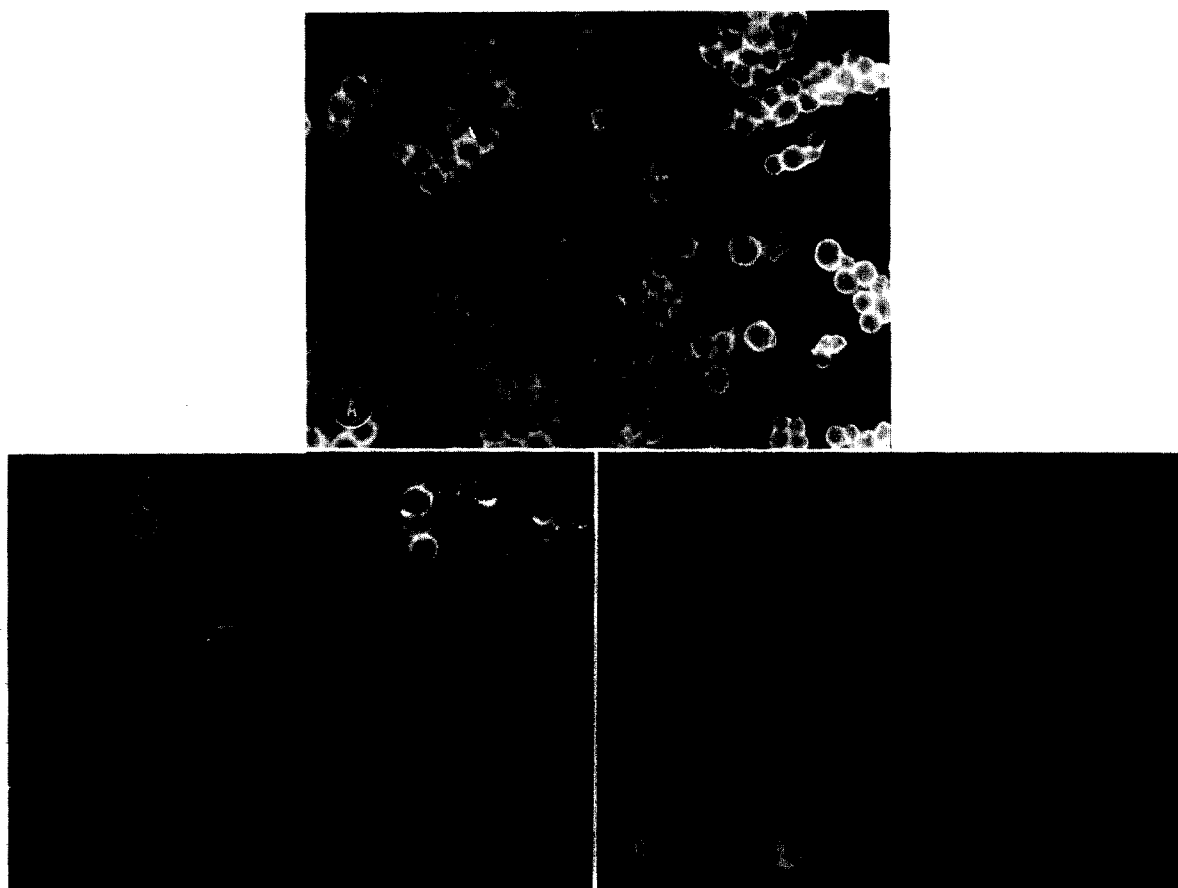


FIG. 4. Photographs of immunofluorescent antibody tests with *A. fumigatus* germlings. A, With antigermling antiserum (1:32). B, With Ag 5 monospecific antiserum (1:32). C, Control test with normal rabbit serum (1:8).

TABLE I. Fluorescent antibody titers to *A. fumigatus* germlings

| Antiserum                               | Titer* |
|-----------------------------------------|--------|
| <i>A. fumigatus</i> -pooled hyperimmune | 512    |
| <i>A. fumigatus</i> spore               | 512    |
| <i>A. fumigatus</i> germlings           |        |
| Initial bleed                           | 1024   |
| Final bleed                             | >2048  |
| Ag 5 monospecific                       | 128    |
| Ag 7 monospecific                       | 8      |
| Ag 13 monospecific                      | 16     |
| <i>Alternaria alternata</i>             | 32     |
| Normal rabbit serum                     | 8-16   |

\*Titers expressed as the reciprocal of highest dilution of antiserum eliciting  $\geq 2+$  fluorescence.

#### Identification of the main germling antigen

By incorporation of the antigermling and monospecific antisera into intermediate gels in self-XRIE test (Fig. 2), the antigen absorbed from the main antibody containing gel was demonstrated, by com-

parison with a standard reference pattern,<sup>7</sup> to be Ag 5 in this system.

In the protein-stained plate incorporating the ABPA pooled serum in the gel, the Ag 5 peak was only poorly visible (Fig. 2, A), whereas in the corresponding autoradiograph (Fig. 2, C), there was a considerable degree of radioactive uptake. ABPA sera therefore contain both precipitating IgG and IgE specific for Ag 5.

Sera from two patients with aspergilloma also had precipitating IgG to Ag 5. In particular, the patient with ABPA, who subsequently developed an aspergilloma, demonstrated intense staining of this component with both IgG (Fig. 3, A and B) and IgE (Fig. 3, C and D), and the level of IgG antibody was also very high, since more antigen and less serum were used in the self-XRIE test for this patient's serum than the ABPA pooled serum (see Fig. 2).

#### Ag 5 as a germling-surface component

**Immunofluorescence assay.** The high titers obtained with antisera produced to culture filtrate, spores, germlings, and Ag 5, compared with the low titers

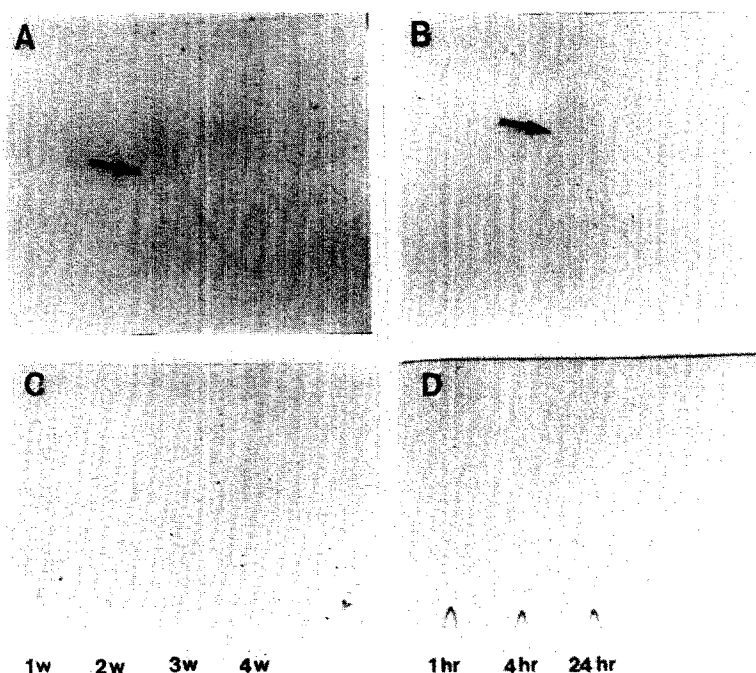


**FIG. 5.** A-B, XIE tests of culture-filtrate antigen (90  $\mu$ g) with Ag 5 monospecific antiserum (15  $\mu$ l/cm<sup>2</sup> gel). A, Control. B, After absorption overnight with formalin-fixed germlings. Increase in height of Ag 5 is indicated. C-D, RIE tests with Ag 5 monospecific antiserum (7.5  $\mu$ l/cm<sup>2</sup> gel). C, Culture filtrate extracts from stationary cultures after 1, 2, 3, and 4 weeks. D, Extracts of spores in HBSS after 1, 4, and 24 hours.

obtained with sera specific for two characterized major antigens, Ag 7 and Ag 13, an *Alternaria alternata* rabbit antiserum and normal rabbit serum are presented in Table I.

Fig. 4 illustrates the appearance of the fluorescence obtained with both the hyperimmune (Fig. 4, A) and Ag 5 monospecific (Fig. 4, B) antisera at 1:32 dilution compared to a control normal rabbit (Fig. 4, C) at 1:8.

**Absorption of monospecific serum.** After absorption of the Ag 5 monospecific antiserum by overnight incubation with washed, formalin-fixed germlings, an increase in the height of the Ag 5 precipitin peak was observed in XIE tests (ie, Fig. 5, B, compared to control [Fig. 5, A]). This indicates a lowering of the antibody titer to Ag 5, since in XIE the height of a peak is inversely related to antibody content. There was no change in antibody titer to Ag 7 after similar incubation of its monospecific antiserum with germlings (not illustrated).

**Solid phase-bound germling ELISA.** Germling-specific IgG levels at a 1:100 dilution of serum are presented in Table II. The antispore serum and the antigerming sera from two rabbits elicited high titers (one, demonstrating a slight reduction after prolonged immunization), as did the Ag 5 monospecific antiserum compared with normal rabbit serum.

**TABLE II.** *A. fumigatus* germling ELISA titers

| Antiserum                                      | Titer* |
|------------------------------------------------|--------|
| <i>A. fumigatus</i> germling A (initial bleed) | 0.69   |
| <i>A. fumigatus</i> germling B (initial bleed) | 1.22   |
| <i>A. fumigatus</i> germling B (final bleed)   | 0.52   |
| <i>A. fumigatus</i> spore antiserum            | 0.75   |
| Ag 5 monospecific antiserum                    | 0.54   |
| Normal rabbit serum                            |        |
| Rabbit A preimmunization                       | 0.23   |
| Rabbit B preimmunization                       | 0.23   |

\*Titers expressed as absorbance at 405 nm obtained with sera diluted 1 in 100.

### Release of Ag 5 from conidia

In RIE tests with monospecific antiserum, Ag 5 was detected in culture-filtrate extracts prepared from both long-term stationary cultures at all culture times (Fig. 5, C), from short-term shake cultures 24 hours after inoculation (not presented), and also in the supernatants of resting conidia after incubation for 1 hour at room temperature in Coca's solution or HBSS (Fig. 5, D).

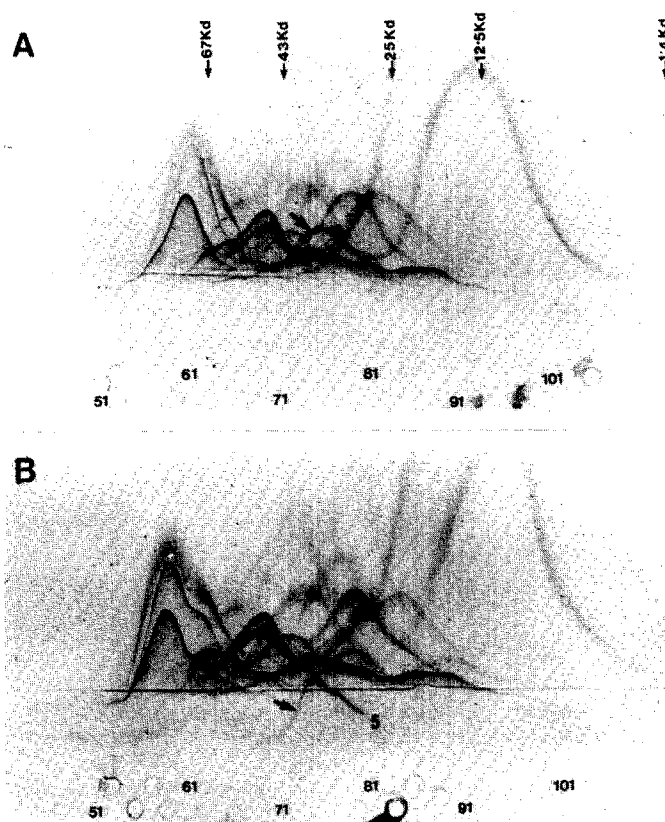


**FIG. 6.** FRIE profile of a Sephacryl S-200 fractionation of protein-enriched culture filtrate extract into reference rabbit antiserum. **A**, Control. **B**, With Ag 5 monospecific antiserum incorporated in an intermediate gel. Positions of molecular weight markers and Ag 5 are indicated.

### Physicochemical characterization of Ag 5

**Molecular weight determination.** By incorporation of the Ag 5 monospecific antiserum in the intermediate gel of a FRIE profile of antigen separated on a calibrated Sephacryl S-200 gel filtration column, Ag 5 was demonstrated to have a molecular weight of approximately 35 kD (Fig. 6).

**Heat stability.** The antigenicity of Ag 5, determined by culture-filtrate antigen and the monospecific antiserum in XIE (Fig. 7, A), was reduced by heating the culture-filtrate antigen at 56° for 30 minutes (Fig. 7, B) and was completely destroyed after heating at 75° for 15 minutes.

**Binding to Con A.** The Ag 5 precipitin peak was not affected by the incorporation of Con A into an intermediate gel XIE test (Fig. 7, C and D).

### DISCUSSION

An antigen present on the surface of germinated conidia, that is, "germlings" of *A. fumigatus*, was investigated by identifying the antigenic component initiating the immunologic response in rabbits immunized with formalin-fixed germinating conidia.

After immunization of rabbits with resting conidia (spores), IgG antibody was detectable, both in an immunofluorescence assay and in an ELISA system in which formalin-fixed germlings were bound to the solid phase of the assay. However, precipitating IgG could not be detected with the antiserum in XIE tests against either culture-filtrate antigen or conidial extracts. These findings are similar to findings reported after the inhalation of aerosolized spores by rabbits.<sup>13</sup> In that study, an indirect ELISA system was able to detect specific IgG in rabbits after only one exposure, whereas precipitating IgG antibodies were not detected in sera obtained from rabbits, even after multiple exposure to spores.

In the present investigation, by contrast, rabbits immunized with germinated conidia (ie, cultured for 15 hours) produced an IgG response, detectable not only by the germling ELISA and immunofluorescence assays but also in XIE gels as an immunoprecipitate to an antigen present in the multicomponent culture-filtrate antigen. In these tests, only one immunoprecipitin peak was observed after the initial immunization, although with repeated immunizations, several

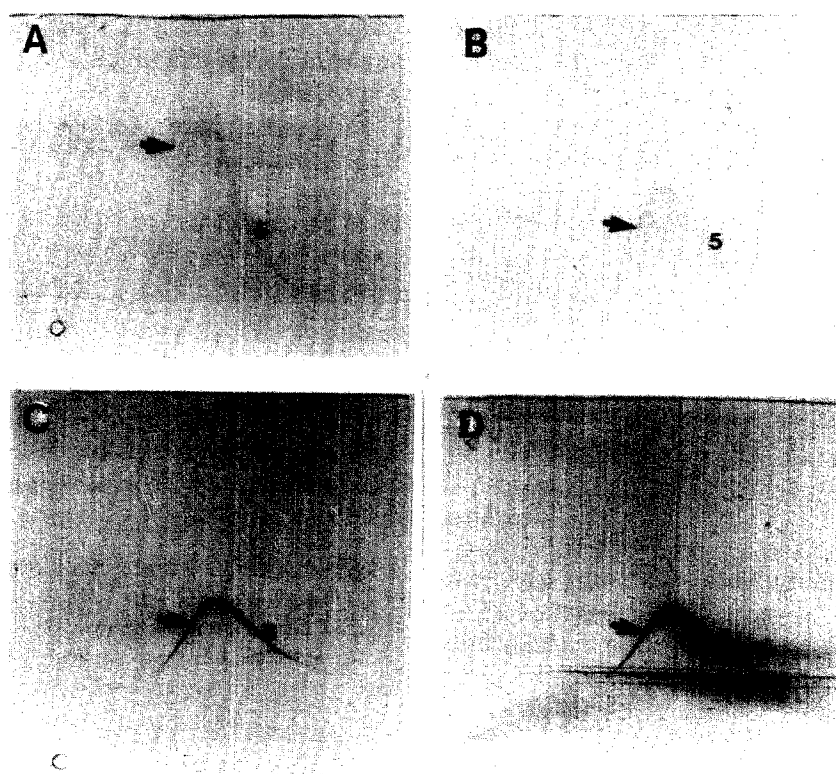


**FIG. 7.** XIE tests of culture filtrate antigen with Ag 5 monospecific antiserum. **A**, Unheated antigen. **B**, After heating at 56° C for 30 minutes. Reduction in Ag 5 peak is indicated. **C**, Control. **D**, Incorporating Con A in an intermediate gel. Height of Ag 5 peak is unaffected.

more immunoprecipitin peaks became visible, which was expected, since precipitating IgG was produced to additional released antigenic components.

As demonstrated for the spore antiserum, other (nonprecipitating) antibodies were also likely to be present in the initial antigermling antiserum. A monospecific antiserum to the "recognized" component was therefore produced for further studies. In particular, the monospecific antiserum was used to identify this component as Ag 5 of the reference self-XRIE pattern of the ABPA serum pool by its incorporation in an intermediate gel.

When the serum IgG titers were compared in the immunofluorescence assay, the initial antigermling serum elicited a higher titer than that obtained with the Ag 5 monospecific antiserum, confirming the premise that IgG antibody reactive with germling-surface components other than Ag 5 was present. Such reactions may not have been observed in the XIE tests performed with the initial antigermling serum (or antispore serum). This could be because either their respective immunoprecipitates in the gel were present in an amount too small to be detected by the relatively insensitive (Coomassie blue) protein stain or they are due to "nonprecipitating" antibody. A third, perhaps

even more likely, explanation is that the antibodies may be directed to surface antigens not present in the culture-filtrate antigen used for the XIE tests and, although they are not further studied here, such antibodies could also be of pathogenic importance.

As the immunofluorescence assay was performed with intact, formalin-fixed conidia, the titers here reflect the presence of antibodies to surface-reactive components, and thus the high titer obtained with the Ag 5 monospecific antiserum indicates the presence of this antigen on the germling surface. By comparison, antisera specific for the characterized major antigens, Ag 7 and Ag 13,<sup>3,4</sup> elicited low titers, further confirming their nature as intracellular antigens, which are released into the culture filtrate only in the third, autolytic phase of growth.<sup>5</sup>

The presence of Ag 5 on the germling surface was further demonstrated, both by the ability of formalin-fixed germlings to absorb Ag 5-specific IgG from the monospecific antiserum and by the relatively high antibody titer obtained with the Ag 5-specific serum in an ELISA system with germlings bound to the solid phase (Table II).

Ag 5 appears to be readily released from the spore surface, since it was present within 1 hour in extracts

from "resting" spores in Coca's solution and HBSS and also in the culture media of shake *A. fumigatus* cultures 24 hours after inoculation. In view of this release of Ag 5 from spores, why Ag 5-specific IgG was produced in rabbits only after immunization with germings and not with spores is not as yet understood.

Antigen 5 was identified in all extracts studied and demonstrated to be a heat-labile protein with a molecular weight, on gel filtration chromatography, of approximately 35 kd. Ag 5 did not bind to the lectin Con A. This is in agreement with previous studies,<sup>12</sup> demonstrating that most of the allergens detected in the self-XRIE system (including Ag 5) were unaffected by this lectin and, hence, lacked terminal  $\alpha$ -D-manno- or  $\alpha$ -D-glucopyranoside sugar residues.

In an investigation into the antigenic and allergenic properties of *A. fumigatus* spore extracts,<sup>12</sup> components released spontaneously from spores into PBS, that is, surface components, demonstrated only low IgE binding in an enzyme allergosorbent test and low activity in skin tests, whereas greater IgE binding and skin test activity occurred with extracts prepared after the mechanical disintegration of spores. This suggested that allergenic/antigenic components might only be released with the destruction of spores after inhalation. However, in the present study, one antigen that appears to be readily released from the spore surface is able to bind both IgE and IgG from ABPA sera.

As demonstrated by self-XRIE tests, sera from patients with ABPA contained both IgG and IgE specific for Ag 5. In a previous study,<sup>7</sup> this component was identified as an allergen, and of 32 patients with ABPA, 38% possessed IgE as well as IgG antibody, since the presence of Ag 5-specific precipitating IgG is implicit in the self-XRIE test used to detect Ag 5-specific IgE. For sera lacking Ag 5 precipitins, specific IgE would not have been revealed, and hence, an even higher percentage of ABPA sera may contain Ag 5-specific IgE.

In the earlier study, the immunoprecipitin peak was not observed in the XIE plate with Coomassie blue staining<sup>7, 14</sup>; however, the observation of the Ag 5 immunoprecipitin peak in self-XRIE tests in the present study with the same reference antigen probably reflects the use of a different ABPA serum pool in which the titer of IgG antibody to Ag 5 was slightly higher. This highlights the problems of detection of precipitating antibody in confirmation of a diagnosis of ABPA and, in particular, the apparent differences sometimes obtained between positive ELISA titers and negative precipitins. As illustrated here, the precipitating antibodies may in fact be present but below

the level of detection by the relatively insensitive techniques as double-diffusion tests with or without protein staining.

It was interesting to note that the sera of two patients with aspergilloma (one associated with ABPA) had very strongly stained, high-titer precipitating antibodies to this component. Higher titer IgG antibodies are probably being produced where the fungus is growing even more actively than in ABPA and thus releasing greater amounts of this antigenic component. This would further suggest that as a rapidly released antigen from the growing fungal surface, Ag 5 may be important not only in triggering immediate reactions in *A. fumigatus*-sensitive subjects but also for detection as a circulating antigen in the serum of patients with invasive aspergillosis.

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## Phenol amplifies complement-fixing activity and induces IgG-precipitating activity in grain-dust extract

Danna Skea, MSc, Donna McAvoy, BSc, and Irvin Broder, MD  
Toronto, Ontario, Canada

*The use of phenol as a preservative in extracts of grain dust was found to be associated with an amplification of complement-fixing activity and the appearance of IgG-precipitating activity. Both activities were absorbed by IgG, suggesting that they were due to a single material. Phenol itself neither fixed complement nor formed a precipitate with IgG. <sup>14</sup>C-labeled phenol was incorporated into the IgG precipitating material. Both the amplification of complement-fixing activity and the appearance of IgG-precipitating activity in phenol-containing grain-dust extract were dependent on the presence of air and were inhibited by heating the extract before phenol addition. One interpretation of these results is that a component of grain-dust extract, possibly an enzyme, oxidatively modifies phenol to a form that fixes complement and precipitates IgG. The modified material demonstrates properties of a polyphenol (tannin). (J ALLERGY CLIN IMMUNOL 1988;81:557-63.)*

Both dry grain dust and extracts prepared from settled grain-elevator dust or from clean crushed grain kernels have been demonstrated to activate the complement system in normal human and guinea pig serum in vitro.<sup>1-5</sup> Complement activation occurred both by the classic and the alternative pathways in the absence of detectable antigrain-dust antibody<sup>4</sup> and resulted in the generation of factors chemotactically active for neutrophils.<sup>3,4</sup> At least some of these studies were performed with extracts of grain dust that contained 0.4% phenol.<sup>5</sup> Phenol was present in the extracts because they were originally prepared for use

### Abbreviation used

PBS: Phosphate-buffered saline

in allergy skin tests, and 0.4% phenol is conventionally used in the preparation<sup>6</sup> and preservation of such reagents (Hollister-Stier Laboratories, Spokane, Wash.). Grain-dust extract containing phenol possessed significantly more complement-fixing activity than grain-dust extract lacking phenol and, in addition, formed a nonimmunologic precipitate with IgG.<sup>5</sup> Freshly prepared phenol extracts contained a low concentration of complement-fixing activity but did not form a precipitate with IgG. Storage for several weeks at 4° C resulted in a large increase in complement fixation and the appearance of IgG-precipitating activity, which could not be explained by the growth of microorganisms. The following study provides evi-

From the Gage Research Institute, Department of Medicine, and Occupational/Environmental Health Unit, University of Toronto, Toronto, Ontario, Canada.

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Reprint requests: I. Broder, MD, The Gage Research Institute, 223 College St., Toronto, Ontario, Canada M5T 1R4.