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An 88-Kilodalton Antigen Secreted by *Aspergillus fumigatus*

HIDEMITSU KOBAYASHI,[1]† JEAN-PAUL DEBEAUPUIS,[1] JEAN-PHILIPPE BOUCHARA,[2] AND JEAN-PAUL LATGE[1]*

Unité de Mycologie, Institut Pasteur, 25 Rue du Dr. Roux, 75015 Paris,[1] and Laboratoire de Parasitologie-Mycologie, Centre Hospitalier d'Angers, 49100 Angers,[2] France

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An 88-kDa component secreted in vitro by *Aspergillus fumigatus* has been purified by sequential chromatographic procedures. The molecule is a glycoprotein with an N-linked sugar moiety composed of mannose glucose, and galactose (16:10:1). It is recognized by antibodies from patients with aspergilloma and has potential for the immunodiagnosis of aspergilloma. The antigenicity is associated with the polypeptide part of the molecule (79 kDa).

Serodiagnostic methods are useful in the detection of aspergilloma (8, 9). With the exception of indirect immunofluorescence tests (7, 22), methods used to detect the presence of specific immunoglobulins to *Aspergillus* species, such as immunodiffusion (14, 19), counterimmunoelectrophoresis (23), and enzyme-linked immunosorbent assay (ELISA) (12, 20), rely on soluble antigens. Most antigenic extracts used routinely in the laboratory are derived from mycelium or culture filtrate and are crude and uncharacterized. These preparations are variable, depending on the origin of the strain, on the growth conditions selected, and on the procedures used to extract the antigenic material (9). Variable findings reported in the serodiagnosis of aspergillosis may also result from the immunological response of the host. Immunoblots have shown individual variations in the specificity of anti-*Aspergillus* immunoglobulins among aspergilloma patients. Moreover, because of the ubiquitous presence of *Aspergillus* spores in the atmosphere, healthy individuals have antibodies directed against most of those antigens which are recognized by antibodies from aspergilloma patients (9, 14).

These variations in the immunoassay results and the proposed future development of automated diagnostic procedures in medical mycology justify the search for a pure antigen which would be indicative of the presence of an aspergilloma. Immunoblot experiments have shown that several antigens are recognized specifically by aspergilloma patients' antibodies (3, 11). However, until now, none of these antigens have been purified. The present study describes the identification, purification, and characterization of an 88-kDa glycoprotein antigen secreted by *Aspergillus fumigatus*. This antigen, recognized by antibodies of aspergilloma patients, is a potential candidate for a standardized immunoassay for the detection of aspergilloma.

MATERIALS AND METHODS

Organisms and culture conditions. Isolates of *A. fumigatus* CBS 144.89, *A. flavus* CBS 569-65, *A. nidulans* CBS 589-65, *A. oryzae* CBS 570-65, and *A. versicolor* CBS 583-65 were used in this study. They were maintained on 2% malt extract agar. *A. fumigatus* was cultivated in three different liquid media: (i) 2% (wt/vol) glucose–1% (wt/vol) mycopeptone (Biokar) (Sabouraud), (ii) 1% yeast extract (Difco), and (iii) 0.2% (wt/vol) collagen. Other species were grown only in 1% yeast extract medium. Flask and fermentor culture conditions were as previously described (14).

Human sera. Serum samples from 72 patients with aspergilloma, 9 patients with allergic bronchopulmonary aspergillosis (ABPA), 22 patients with candidiasis, and 28 healthy individuals were used in this study. Aspergilloma was diagnosed on the basis of X-ray analysis, clinical examination, serological reactions, and isolation of *A. fumigatus* from sputa or bronchial washings. ABPA patients were chosen among patients without cystic fibrosis. For these patients, positive serology and *A. fumigatus* isolation were always associated with hypereosinophilia and an increase in total immunoglobulin E (IgE). Serum antibody reactivity was monitored by both Ouchterlony diffusion assays using somatic and metabolic extracts from *A. fumigatus* (Diagnostic Pasteur, Marnes, France) and passive hemagglutination assays using the kit from Fumouze (Clichy, France) (6, 17). The sera from the candidiasis patients were negative by these assays. Human positive and negative serum pools contained equal volumes of 50 different serum samples.

Rat monoclonal antibody. A rat IgM monoclonal antibody (EB-A2) to *A. fumigatus* galactomannan was a gift from D. Stynen (Diagnostics Pasteur, Genk, Belgium). This monoclonal antibody recognizes the side chains containing β-(1→5)-galactofuranosyl residues of galactomannan (21).

Extraction of antigen. Culture filtrate was concentrated (10-fold) by ultrafiltration on a Pellicon cassette (Millipore Corp., Bedford, Mass.). The concentrate was precipitated with 4 volumes of ethanol overnight at 4°C. The ethanol precipitate was washed twice with ethanol, resuspended in 20 mM Tris-HCl (pH 8.8) at 10 to 20 mg (dry weight) of ethanol precipitate per ml, ultrasonicated for 1 min (Sonifer cell disrupter B3D; 600 W; microtip probe diameter, 3 mm), and further incubated for 30 min at room temperature. The undissolved precipitate was removed by centrifugation, and the water-soluble material from the ethanol precipitate was aliquoted and stored at −20°C for further analysis. The water-soluble materials obtained from the three different media, Sabouraud, yeast extract, and collagen, were designated as WSEP-S, WSEP-Y, and WSEP-C, respectively.

Chromatographic purification of the 88-kDa component. WSEP-Y was filtered through a 0.2-μm-pore-size Minisart N filter unit (Sartorius, Göttingen, Germany). The filtrate (50 ml; 0.2 g) was loaded on a Sartobind-Q anion-exchange

* Corresponding author.
† Present address: Second Department of Hygienic Chemistry, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Sendai Aobaku, Miyagi 981, Japan.

membrane (Sartorius) washed previously with 5 ml of 20 mM Tris-HCl (pH 8.8). The membrane was washed twice with the same buffer and then with 1 ml of 1 M sodium acetate to elute the bound proteins. The eluate was dialyzed overnight against water at 4°C. After concentration under vacuum to 200 μl, the sample was applied to a gel filtration Superdex 75 column (HR 10/30, Pharmacia high-performance liquid chromatography [HPLC] column). The elution buffer was 20 mM Tris-HCl (pH 8.8) containing 0.15 M sodium acetate, with a flow rate of 0.8 ml/min. Proteins in the molecular mass range of 85 to 90 kDa were concentrated and dialyzed on a Centricon ultrafiltration cell with a molecular mass cutoff of 30 kDa (Amicon, Danver, Mass.).

The desalted fraction was adjusted to pH 8.8 with Tris-HCl buffer to a final concentration of 20 mM, filtered by using a 0.2-μm-pore-size filter unit (type HV; Nihon Millipore Kogyo, Yonezawa, Japan), and applied to an anion-exchange HPLC column (ProPac PA1, 4 by 250 mm; Dionex). Elution was performed with a gradient of sodium acetate (0 to 500 mM in 30 min at 0.8 ml/min). During all chromatographic procedures, the protein content was estimated by measuring UV A_{278}. The presence and the purity of the 88-kDa molecule were monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.

Treatment of the 88-kDa component with PNGase F. A dry sample of the 88-kDa molecule (20 μg) was solubilized in 20 μl of buffer (20 mM phosphate [pH 7.5], 50 mM EDTA, 0.02% [wt/vol] sodium azide, 50% glycerol), supplemented with 0.5% (wt/vol) SDS and 5% (vol/vol) β-mercaptoethanol. The sample was heated at 100°C for 2 min. After the sample was cooled, 5% Nonidet P-40 and 5 U of peptide–*N*-glycosidase F (PNGase F; Oxford GlycoSystems, Abingdon, United Kingdom) were added, and the mixture was incubated at 37°C for 24 h.

Deglycosylation was assessed by using protocol 1a of the Glycotrack kit (Oxford GlycoSystems). Basically, after transfer of the protein onto an Immobilon membrane, the protein was oxidized with 10 mM sodium periodate, incubated successively with biotin-hydrazide and streptavidin-alkaline phosphatase, and finally stained with a mixture of 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium.

Sugar analysis of the 88-kDa component. Trimethylsilylation of monosaccharides released after overnight methanolysis at 80°C of the 88-kDa molecule in 0.5 N HCl-methanol was performed as previously described (15). Separation of the trimethylsilylated methylglycoside was performed by gas-liquid chromatography on a capillary methylsilicone DB-1 column (J&W Scientific, Folsom, Calif.) with N_2 as the carrier gas at 1 bar (10^5 Pa) and the oven temperature programmed for 120 to 200°C at a rate of 2°C/min.

SDS-PAGE and isoelectric focusing. Samples were solubilized at 100°C for 3 min in a buffer containing 0.5% (wt/vol) SDS and 1.25% (vol/vol) mercaptoethanol. Electrophoresis was done by using the discontinuous buffer system of Laemmli (13) in 7.5 or 12% acrylamide gel slabs. Gels were stained by the silver nitrate method (2). The M_rs of the *Aspergillus* proteins were calculated on a 7.5% acrylamide gel slab by comparison with standards from a low-molecular-weight electrophoresis calibration kit from Pharmacia.

Isoelectric focusing was performed by using Pre Net acrylamide film (Serva), with a pH range of 3 to 10 and a deposit of a 10-μl sample of the desalted 88-kDa protein. Running conditions and Coomassie blue staining were as recommended by the manufacturer (Serva).

Immunoblotting. After electrophoresis, samples were electrotransferred onto nitrocellulose membranes and immunoblotted as previously described (14).

Immunoblot experiments were performed with the following antigens: WSEP (25 μg of protein per well) from all culture media and the purified 88-kDa component and its deglycosylated form (each at 0.2 μg of protein per well). Sera from aspergilloma patients and healthy individuals (each at a 1:1,000 dilution) alone or EB-A2 rat antigalactomannan monoclonal antibody (at a 1:2,000 dilution, 2.5 μg of IgM per ml) were used. Peroxidase-conjugated antibodies (anti-human and anti-rat IgG whole molecules) were diluted 1:1,000.

Isolation of anti-88-kDa-protein-specific antibodies. A sample of purified 88-kDa protein (5 μg per 10-cm-long well) was electrophoresed and electrotransferred onto a nitrocellulose membrane. After staining of the blot with Ponceau red S, the band corresponding to the 88-kDa protein was cut out of the blot and blocked in TBSTM buffer (0.1 M Tris-HCl [pH 8.0], 0.15 M NaCl, 5 mM Na_3-EDTA, 0.1% Tween 20, 10% [vol/vol] defatted milk). After 2 h at room temperature, a pool of human sera reacting positively with this protein was diluted 1:100 in TBSTM and incubated with the nitrocellulose band. After 2 h at room temperature, the blot was washed three times in TBSTM without milk and twice in Tris buffer containing 0.15 M NaCl. Anti-88-kDa-protein-specific antibodies were removed from the blot by a 30-min incubation in Tris buffer (pH 8.0) containing 1 M NaCl. The incubation buffer was dialyzed for 24 h against distilled water, concentrated under vacuum, and stored at −20°C. The specificity of the antibodies removed from the blot was verified by the labelling of a single band at 88 kDa when a blot from a total extract from WSEP-Y medium was incubated with the antibody solution.

ELISA. Wells of polyvinyl microtiter plates (reference no. 655101; Greiner Labortechnik, Frichkenhausen, Germany) were coated with 0.25 μg of the 88-kDa protein per ml in 50 mM carbonate buffer (pH 9.6) and incubated for 1 h at 37°C and overnight at 4°C. Human sera (in 100 μl aliquots from 40 aspergilloma patients, 20 healthy individuals, and 20 candidiasis patients) were diluted 1:500 in phosphate-buffered saline containing 0.5% (vol/vol) Tween 20 and 1% (wt/vol) bovine serum albumin. Quantification of the immunoglobulins bound to the protein antigen was done by using peroxidase-conjugated antibodies (anti-human IgG whole molecule; Biosys, Compiègne, France) diluted 1:1,000 (14).

RESULTS

Antigenicity of water-soluble components secreted by *A. fumigatus* in three different culture media. SDS-PAGE of ethanol precipitate extracts from the culture media showed that the patterns of proteins secreted by *A. fumigatus* differed with the culture medium (Fig. 1A). Major proteins in WSEP-S had apparent molecular weights of 18,000 to 94,000. WSEP-C contained mainly high-molecular-weight bands ranging from 30,000 to 88,000. WSEP-Y proteins had bands intermediate between those in the two other media.

Immunoblot analysis with aspergilloma patients' sera showed that most of the proteins which stained with silver nitrate were antigenic. The major antigens in WSEP-S were the doublet at 18 and 28 kDa previously characterized (14). A minor band at 88 kDa was also present. This band represented the major component in both yeast extract and collagen media (Fig. 1B).

Purification of the 88-kDa component. While the 88-kDa component is a major protein in both yeast extract and

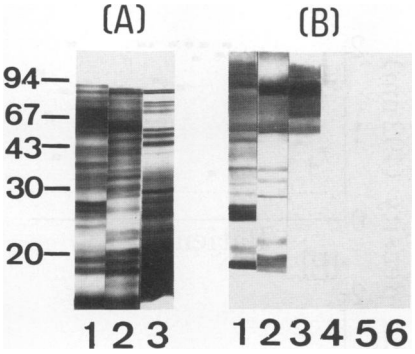

FIG. 1. (A) SDS-PAGE protein patterns of WSEP-S (lane 1), WSEP-Y (lane 2), and WSEP-C (lane 3) after silver nitrate staining; (B) immunoblot analysis of WSEP-S (lanes 1 and 4), WSEP-Y (lanes 2 and 5), and WSEP-C (lanes 3 and 6) using a 1:1,000 dilution of human positive (lanes 1 to 3) and negative (lanes 4 to 6) serum pools. Molecular mass markers (in kilodaltons) are shown on the left.

collagen media, purification of this antigen was performed in WSEP-Y because it was more easily handled in liquid chromatography than WSEP-C.

HPLC on a Superdex 75 column of the fraction that bound to the Sartobind-Q membrane showed a major peak corresponding to proteins with molecular masses of 85 to 90 kDa (Fig. 2). This peak accounted for 69% of the total protein eluted from the column. This fraction was loaded on a ProPac PA1 anion-exchange column. Elution of this fraction gave a major peak at 0.23 M sodium acetate (Fig. 3). This peak, which accounted for 71% of the total eluted protein, contained a pure 88-kDa protein (Fig. 4A, lane 2). Isoelectric focusing experiments showed that this protein was composed of three isoelectric variants with pIs

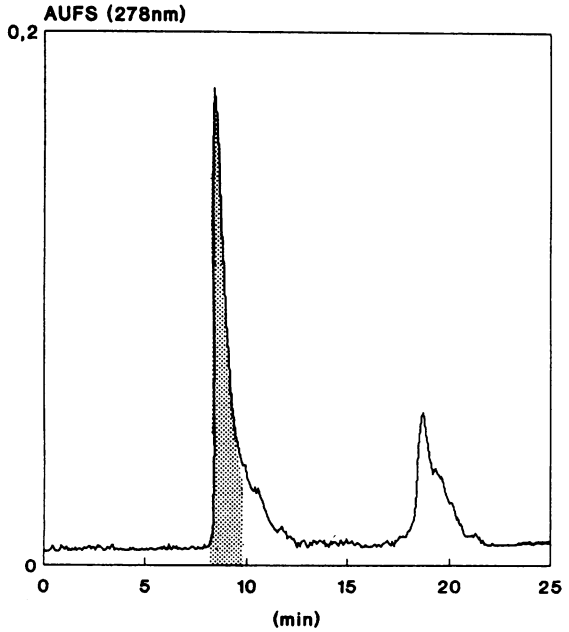

FIG. 2. Superdex 75 HPLC elution profile of the Sartobind-Q-bound fraction of WSEP-Y. The shaded area indicates the peak corresponding to 85- to 90-kDa proteins. A flow rate of 0.8 ml/min in elution buffer (20 mM Tris-HCl [pH 8.8] with 0.15 M sodium acetate) was used. AUFS (278 nm), A_{278}.

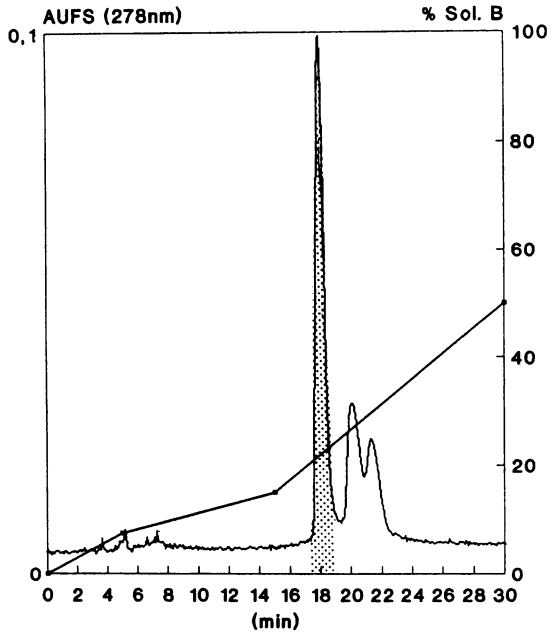

FIG. 3. ProPac PA1 HPLC elution profile of the peak recovered (see Fig. 2). The shaded area indicates the peak at which the 88-kDa component is recovered. Sol. B, solution B (20 mM Tris-HCl [pH 8.8] with 1 M sodium acetate); AUFS (278 nm), A_{278}.

of 5.7 to 5.9 (data not shown). The value of this pI was in agreement with the efficient binding of this protein to a strong anion-exchange column.

Characterization of the 88-kDa component. The 88-kDa component was glycosylated as indicated by a positive reaction with the Glycotrack carbohydrate detection kit (Fig. 4B, lane 1). Gas-liquid chromatography analysis showed that the sugar moiety of this antigen was composed of mannose, glucose, and galactose in the ratio of 16:10:1 (data not shown). This glycoprotein showed no reactivity with the antigalactomannan monoclonal antibody, EB-A2, indicating that the carbohydrate moiety of the 88-kDa com-

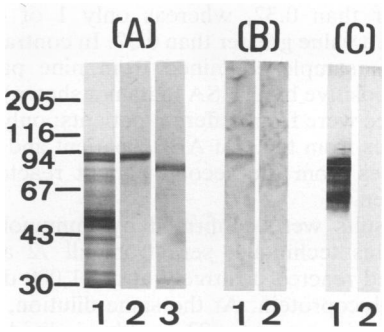

FIG. 4. Characterization of the 88-kDa component and its deglycosylated molecule. (A) SDS-PAGE of WSEP-Y (lane 1), the 88-kDa molecule (lane 2), and its deglycosylated form (lane 3) after Coomassie blue staining; (B) detection of sugar residues of the 88-kDa molecule (lane 1) and its deglycosylated form (lane 2) by using Glycotrack; (C) immunoblot analysis of WSEP-Y (lane 1) and the 88-kDa molecule (lane 2) using a 1:2,000 dilution of the antigalactomannan monoclonal antibody (EB-A2). Molecular mass markers (in kilodaltons) are shown on the left.

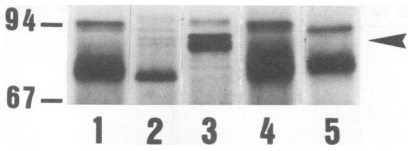

FIG. 5. SDS-PAGE protein patterns of extracts from *A. flavus* (lane 1), *A. nidulans* (lane 2), *A. fumigatus* (lane 3), *A. oryzae* (lane 4), and *A. versicolor* (lane 5). The window shown corresponded to molecular masses of 94 to 67 kDa (indicated on the left) in a 7.5% acrylamide slab gel. The 88-kDa antigen is indicated by an arrowhead.

ponent did not contain β-(1→5)-galactofuranosyl residues (Fig. 4C, lane 2).

When a purified 88-kDa component was treated with PNGase F and submitted to SDS-PAGE, two bands with apparent molecular weights of 79,000 and 35,000 were detected by silver staining (Fig. 4A, lane 3). The small component corresponds to PNGase F. This result indicated that a 9-kDa-carbohydrate domain was eliminated from the 88-kDa antigen by deglycosylation treatment. The 79-kDa band reacted negatively with the Glycotrack test, indicating that the PNGase F digestion was complete (Fig. 4B, lane 2). This result showed also that the sugar moiety was exclusively N linked to the protein.

When extracts of different species of *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. oryzae*, *A. nidulans*, and *A. versicolor*) were submitted to SDS-PAGE and stained with silver nitrate, *A. fumigatus* was the only species that secreted the 88-kDa protein (Fig. 5). The absence of the 88-kDa antigen was confirmed by immunoblot analysis of total extracts of the different species of *Aspergillus* probed with specific anti-88-kDa-protein antibodies isolated as described in Materials and Methods. Only one band with an M_r of 88,000 was labelled in the lane containing *A. fumigatus* (data not shown).

Antigenicity of the 88-kDa glycoprotein and its deglycosylated 79-kDa component. ELISA values obtained from a total of 40 individual human serum samples from patients with aspergilloma and 40 negative serum samples reacting with the 88-kDa glycoprotein are shown in Fig. 6. An optical density of 0.32 corresponds to the mean + 4 standard deviations calculated for 40 serum samples from 20 healthy individuals and 20 patients with candidiasis. All 40 serum samples from patients with aspergilloma gave optical density values higher than 0.32, whereas only 1 of 40 negative controls gave a value greater than 0.32. In contrast, only 2 of the 31 serum samples obtained from nine patients with ABPA were positive by ELISA (data not shown). These two serum samples were from different patients; only one of two serum samples from the first ABPA patient and one of nine serum samples from the second patient reacted with the 88-kDa antigen.

ELISA results were confirmed by immunoblot experiments. By this technique, sera from all 72 aspergilloma patients tested reacted positively at a 1:1,000 dilution with the 88-kDa glycoprotein. At the same dilution, 92% of the sera from the 50 controls (22 healthy individuals and 28 candidiasis patients) did not show the 88-kDa band, whereas the remaining 8% of the sera gave a very-low-intensity band with this protein (data not shown).

Immunoblotting experiments with the 88-kDa glycoprotein and its deglycosylated 79-kDa protein moiety with 5 individual serum samples and the pool of positive sera from patients with aspergilloma showed no demonstrable decrease in the intensity of the immunoreactive band following deglycosylation with PNGase F (Fig. 7). This indicates that the sugar moiety does not bear the antigenic epitopes of the 88-kDa glycoprotein.

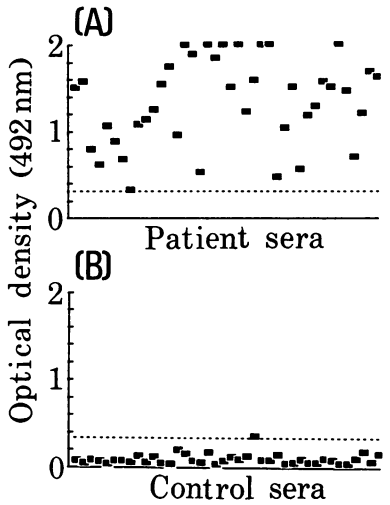

FIG. 6. Results at an optical density of 492 nm obtained in ELISA experiments with the 88-kDa antigen reacted with 40 serum samples from aspergilloma patients (A) and 40 negative serum samples (from 20 healthy individuals and 20 candidiasis patients) (B). All sera were used at a 1:500 dilution, and microtiter plates were coated with 0.25 μg of 88-kDa antigen per ml.

DISCUSSION

Most of the antigens with a molecular mass greater than 35 kDa secreted by *A. fumigatus* are glycoproteins (14). The majority of these antigens bound both to concanavalin A and antigalactofurane monoclonal antibodies (10, 11, 14, 21). The results indicated that the sugar moiety of these molecules is composed of a galactomannan with galactofuranosyl side chains which are responsible for their antigenicity (21). Fractions containing concanavalin A-binding antigens are usually the most reactive antigens and have been used in the diagnosis of aspergillosis (8). However, to date, only two glycopeptide antigens have been purified. The first one is a 58-kDa concanavalin A-binding molecule containing 46%

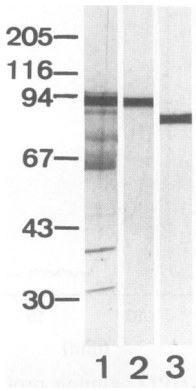

FIG. 7. Immunoblot analysis of the WSEP-Y (lane 1), the 88-kDa antigen (lane 2), and its deglycosylated molecule (lane 3) using a 1:1,000 dilution of a pool of sera from aspergilloma patients. Molecular mass markers (in kilodaltons) are indicated on the left.

(wt/wt) carbohydrate with a Man-Gal-Glc ratio of 2:1:1. This antigen is thought to be O linked to the protein, as indicated by the presence of relatively high quantities of serine and threonine residues (5). The second one is a 20-kDa allergen which does not bind to concanavalin A and has a sugar moiety with a Man-Gal-Glc ratio of 2:1:0.5 (18). The possible immunoreactivity of the sugar moiety of these two antigens has not been investigated. In this study, we isolated an 88-kDa glycoprotein which contains only N-linked sugars. In contrast to the 20- and 58-kDa glycoproteic antigens, the 88-kDa protein contained a very low amount of galactose. In addition, the inability of this antigen to bind to a monoclonal antibody specific to β-(1→5)-galactofuranosyl oligomers indicates that the structure of the sugar entity is markedly different from that of the galactomannan reported as being present in many glycoproteic antigens of *A. fumigatus*. Deglycosylation experiments which have shown that the polypeptide part of the 88-kDa antigen bears the antigenic binding sites confirmed the absence of galactofuran in this antigen.

Antigenic proteins with a similar molecular mass (86 to 90 kDa) have been observed previously by immunoblotting after separation by SDS-PAGE (5, 18, 24). With the exception of the 88-kDa antigen described by Burnie and Matthews (3), none of these antigens have been purified or characterized. The 88-kDa antigen studied by Burnie and Matthews belongs to the heat shock protein 90 family. Microsequencing results of N-terminal and two internal peptides (obtained following tryptic digestion of the 88-kDa antigen) showed no homology between it and *Saccharomyces cerevisiae* heat shock protein 90 (unpublished data). Moreover, sera from patients with *Candida* infections did not react with our antigen. These results suggest that the 88-kDa protein that we isolated from *A. fumigatus* does not belong to the heat shock protein 90 family.

This study confirmed previous reports reviewed by Hearn (9) showing that the composition of the culture medium greatly influences the pattern of antigens secreted by *A. fumigatus*. A medium based on protein or a protein hydrolysate in the absence of sugar supplementation may more closely reflect the nutrient conditions found by the fungus in its host. Moreover, during growth in a 1% yeast extract or 0.2% collagen medium, the pH remains neutral or slightly basic (7 to 7.5) whereas it becomes acidic (4 to 5) and far removed from physiological conditions in medium such as Sabouraud or Czapek with a 2 or 3% sugar supplementation (16). The use of nitrogen-rich media should be recommended to obtain the secretion of an antigen library similar to the ones produced in vivo.

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REFERENCES

1. **Bernstein, J. A., C. R. Zeiss, P. A. Greenberger, R. Patterson, J. F. Marhoul, and L. L. Smith.** 1990. Immunoblot analysis of sera from patients with allergic bronchopulmonary aspergillosis: correlation with disease activity. J. Allergy Clin. Immunol. **86**:532–539.
2. **Blum, H., H. Beier, and H. J. Gross.** 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8**:93–99.
3. **Burnie, J. P., and R. C. Matthews.** 1991. Heat-shock protein 88 and *Aspergillus* infection. J. Clin. Microbiol. **29**:2099–2106.
4. **Coleman, R. M., and L. Kaufman.** 1972. Use of the immunodiffusion test in the serodiagnosis of aspergillosis. Appl. Microbiol. **23**:301–308.
5. **Fratamico, P. M., and H. R. Buckley.** 1991. Identification and characterization of an immunodominant 58-kilodalton antigen of *Aspergillus fumigatus* recognized by sera of patients with invasive aspergillosis. Infect. Immun. **59**:309–315.
6. **Gold, J. W. M., B. Fisher, B. Yu, N. Chein, and D. Armstrong.** 1980. Diagnosis of invasive aspergillosis by passive hemagglutination assay of antibiotics. J. Infect. Dis. **142**:87–94.
7. **Gordon, M. A., E. W. Lapa, and J. Kane.** 1977. Modified indirect fluorescent-antibody test for aspergillosis. J. Clin. Microbiol. **6**:161–165.
8. **Hearn, V. M.** 1988. Serodiagnosis of aspergillosis, p. 43–71. *In* H. Vanden Bossche, D. W. R. Mackenzie, and G. Cauwenbergh (ed.), *Aspergillus*, aspergillosis. Plenum Press, New York.
9. **Hearn, V. M.** 1992. Antigenicity of *Aspergillus* species. J. Med. Vet. Mycol. **30**:11–25.
10. **Hearn, V. M., B. L. Griffiths, and P. A. J. Gorin.** 1989. Structural analysis of water-soluble fractions obtained from *Aspergillus fumigatus* mycelium. Glycoconjugate J. **6**:85–100.
11. **Hearn, V. M., E. V. Wilson, J.-P. Latgé, and D. W. R. Mackenzie.** 1990. Immunochemical studies of *Aspergillus fumigatus* mycelial antigens by polyacrylamide gel electrophoresis and Western blotting techniques. J. Gen. Microbiol. **136**:1525–1535.
12. **Holmberg, K., M. Berdischewsky, and L. S. Young.** 1980. Serological diagnosis of invasive aspergillosis. J. Infect. Dis. **141**:656–664.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Latgé, J.-P., M. Moutaouakil, J. P. Debeaupuis, J. P. Bouchara, K. Haynes, and M.-C. Prévost.** 1991. The 18-kilodalton antigen secreted by *Aspergillus fumigatus*. Infect. Immun. **59**:2586–2594.
15. **Montreuil, J., S. Bouquelet, H. Debray, B. Fournet, G. Spik, and G. Strecker.** 1986. Glycoproteins, p. 143–204. *In* M. F. Champlin and J. F. Kennedy (ed.), Carbohydrate analysis: a practical approach. IRL Press, Oxford.
16. **Moutaouakil, M., M. Monod, M. C. Prévost, J. P. Bouchara, and J. P. Latgé.** Identification of the 33-kDa alkaline protease of *Aspergillus fumigatus* in vitro and in vivo. J. Med. Microbiol., in press.
17. **Philpot, C. M., and D. W. R. Mackenzie.** 1976. A comparison of antibodies to *Aspergillus fumigatus* in agar gel with different antigens and immunodiffusion patterns. J. Biol. Stand. **4**:73–79.
18. **Samuelsen, H., A. Karlsson-Borga, B. S. Paulsen, J. K. Wold, and W. Rolfsen.** 1991. Purification of a 20 kD allergen from *Aspergillus fumigatus*. Allergy **46**:115–124.
19. **Schaefer, J. C., B. Yu, and D. Armstrong.** 1976. An aspergillosis immunodiffusion test in the early diagnosis of aspergillosis in acute leukemia patients. Am. Rev. Respir. Dis. **113**:325–329.
20. **Schönheyder, H., and P. Andersen.** 1984. IgG antibodies to purified *Aspergillus fumigatus* antigens determined by enzyme-linked immunosorbent assay. Int. Arch. Allergy Appl. Immunol. **74**:262–269.
21. **Stynen, D., J. Sarfati, A. Goris, M.-C. Prévost, M. Lesourd, H. Kamphuis, V. Darras, and J.-P. Latgé.** 1992. Rat monoclonal antibodies against *Aspergillus* galactomannan. Infect. Immun. **60**:2237–2245.
22. **Warnock, D. W.** 1974. Indirect immunofluorescence test for the detection of *Aspergillus fumigatus* antibodies. J. Clin. Pathol. **27**:911–912.
23. **Wilson, E. V., and V. M. Hearn.** 1983. Use of *Aspergillus fumigatus* mycelial antigens in enzyme-linked immunosorbent assay and counter-immunoelectrophoresis. J. Med. Microbiol. **16**:97–105.
24. **Yu, B., Y. Niki, and D. Armstrong.** 1990. Use of immunoblotting to detect *Aspergillus fumigatus* antigen in sera and urines of rats with experimental invasive aspergillosis. J. Clin. Microbiol. **28**:1575–1579.