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Binding of Host Collectins to the Pathogenic Yeast *Cryptococcus neoformans*: Human Surfactant Protein D Acts as an Agglutinin for Acapsular Yeast Cells

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***Cryptococcus neoformans* is an opportunistic pathogen in AIDS patients causing disseminated disease and lethal meningitis after inhalation of acapsular or sparsely encapsulated yeast cells. In this study we have investigated whether a recently described family of primitive opsonins, termed collectins, contribute to innate resistance against *C. neoformans*. The pulmonary surfactant proteins SP-A and SP-D as well as the serum collectins mannose-binding protein and CL-43 bound in a calcium-dependent manner to acapsular *C. neoformans* in vitro. Binding was concentration dependent and abolished by competition with defined mono- and oligosaccharides. In contrast, no binding of the collectins was observed with the encapsulated form of the yeast. Furthermore, binding of purified collectin SP-D, but not SP-A, mannose-binding protein, or CL-43, led to a concentration-dependent agglutination of acapsular *C. neoformans*. These data indicate that collectins recognize carbohydrate structures in the cell wall of an initial infectious form of *C. neoformans* and may play a role in early antifungal defenses in the lung.**

Cryptococcus neoformans, a basidiomycetous yeast, has a worldwide distribution and is an increasingly important opportunistic pathogen in AIDS patients. Infection is believed to occur by inhalation of acapsular yeast cells or basidiospores found in soil enriched in pigeon droppings (21). In the immunocompetent host, the infection is controlled by the local pulmonary immune mechanisms. In contrast, in the absence of adequate cell-mediated immunity, the organism disseminates to other tissues such as bone, skin, and viscera and, in particular, to the brain, causing lethal meningoencephalitis. There is currently no curative treatment, and even with aggressive chemotherapy the mortality rates among immunocompromised hosts are high (16). Pulmonary immune defenses therefore play an important role in controlling the initial growth of the organism and preventing further dissemination, but the mechanisms which regulate immunity at this site remain poorly understood. Most studies on the immunobiology of cryptococcosis have focussed on encapsulated yeast cells which can be found in lung tissue within 5 to 10 h after initial contact with acapsular organisms (6). Induction of capsule synthesis is a major virulence factor for *C. neoformans* and is correlated both in animal models and in the clinical setting with productive infection (28). The primary action of the capsule is to prevent ingestion of the organism by host phagocytes, and the polysaccharide structure has also been reported to have immunomodulatory properties including the suppression of specific antibody production and the induction of T-cell tolerance (20). The antiphagocytic action of the capsule can be partially overcome by opsonization with either specific immunoglobulins or complement components, such as C3b and iC3b, allowing ingestion of the organism by macrophages (17). Furthermore, our laboratory has demonstrated that complement-dependent phagocytosis of *C. neoformans* can be strongly enhanced if macrophages are activated by tumor necrosis factor alpha and granulocyte-macrophage colony-stimulating factor and that in vivo production of these two cytokines is essential for resistance in a murine model of infection (2).

Little is known about the mechanisms of immunity to acapsular yeast cells, which together with basidiospores represent the most likely infectious forms of *C. neoformans* for the host. Acapsular *C. neoformans* possesses a typical fungal cell wall enriched in glucan and galactoxylomannan and is readily ingested by murine and human macrophages (28). We have recently demonstrated that phagocytosis of acapsular yeast cells by murine macrophages is effectively inhibited in vitro by purified mannan and β-1,3-glucan, implicating an involvement of the macrophage mannose and β-glucan receptors in this process (3). In addition, the acute-phase reactant mannose-binding protein (MBP), which displays binding specificity similar to that of the mannose receptor, also binds to the acapsular yeast cell in vitro (3). MBP is a member of a family of proteins termed collectins, which include the lung surfactant proteins A and D (SP-A and SP-D, respectively) (13, 25). This family also includes the serum proteins collectin-43 (CL-43) and bovine conglutinin (BK), which to date have been identified only in cattle (13). Collectins belong to the animal C-type lectin superfamily characterized by a carbohydrate recognition domain (CRD) which binds ligands in a Ca^{2+}-dependent manner plus a collagen tail involved in their biological function (13, 26). Low levels of MBP in the serum are associated with increased infection during childhood, and there is now increasing evidence that the surfactant proteins SP-A and SP-D are involved in early pulmonary defenses against pathogens and foreign

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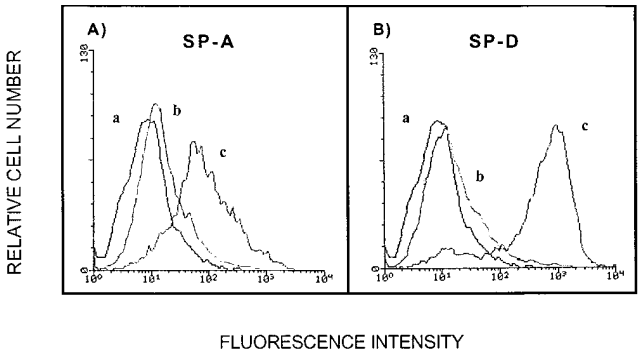

FIG. 1. Flow cytometric analysis of binding of SP-A and SP-D to acapsular *C. neoformans*. The collectins were incubated with 10^6 *C. neoformans* cells in calcium containing buffer and stained with rabbit anticollectin antibody followed by goat anti-rabbit Ig–FITC-labelled antibody as described in the text. FACScan analysis of 5,000 yeast cells is shown for unstained *C. neoformans* (a), *C. neoformans* with rabbit anticollectin, goat anti-rabbit Ig–FITC-labelled antibody (b), and *C. neoformans* with collectin plus rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies (c). The data show results of a representative experiment of 10 for SP-A and of 9 for SP-D.

particles (7, 24, 27, 38). Nevertheless, the range of pulmonary pathogens recognized by SP-A or SP-D and the effect of opsonization by these proteins on the interaction with host phagocytes have not been adequately defined. The aim of this project was to examine binding of purified collectins to the human opportunistic pathogen *C. neoformans*. Using FACScan analysis, we have demonstrated specific and calcium-dependent binding of human SP-A, SP-D, and the serum collectins MBP and CL-43 to acapsular yeast cells. Furthermore, binding of SP-D resulted in agglutination of acapsular, but not encapsulated, *C. neoformans* in vitro. These results raise the possibility that surfactant proteins SP-A and SP-D may play an important role in innate resistance against fungal pathogens.

MATERIALS AND METHODS

C. neoformans. Encapsulated *C. neoformans* var. *neoformans* (*C. neoformans*) isolate B3501 and its coisogenic acapsular mutant B4131 were used throughout these studies. The organisms were grown on Sabouraud dextrose agar at 37°C, harvested after 48 to 72 h, washed twice in pyrogen-free saline, and stored in pyrogen-free saline at 4°C. The presence or absence of capsule synthesis by these strains was determined by immunofluorescence analysis. Incubation of 10^6 yeast cells with 100 μl of rabbit anti-*C. neoformans* capsule antibody (Alpha Laboratories, Hampshire, United Kingdom) diluted in phosphate-buffered saline (PBS) containing 10% fetal calf serum (Gibco, Paisley, United Kingdom) was followed by incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobin (Ig) diluted 1:200 (Sigma, Dorset, United Kingdom). Yeast cells were analyzed by flow cytometry with a Becton Dickinson FACScan with LYSIS II software. To confirm capsule size, an India ink test was performed by mixing 20 μl of yeast cells diluted in pyrogen-free saline with 20 μl of India ink solution, and the mixture was then placed on a slide with a coverslip and examined by light microscopy. All organisms were kindly provided by K. J. Kwon-Chung, National Institutes of Health, Bethesda, Md.

Purification of collectins and antisera. Human SP-A was isolated from lung lavage fluids of healthy volunteers and purified as described previously (11). Human SP-D was isolated from a patient with alveolar proteinosis as described previously (12). Human MBP was isolated and purified from serum as described previously (23), and bovine CL-43 was isolated and purified as described previously (12).

Polyclonal rabbit antisera to MBP and SP-A were kindly provided by J.-C. Jensenius, S. Thiel, and colleagues (University of Aarhus, Aarhus, Denmark). Polyclonal rabbit anti-SP-D antibody was a gift from J. Lu (University of Oxford, Oxford, United Kingdom).

Determination of collectin binding to *C. neoformans*. The following method describing collectin binding to *C. neoformans* was used for all collectins tested. Acapsular *C. neoformans* (10^6 organisms) was washed twice in filtered PBS containing 1% bovine serum albumin and 1.5 mM $CaCl_2$ (PBS-BSA-Ca^{2+}; pH 7.4) and centrifuged (Sorvall, RT 6000D [Du Pont]) at 2,000 rpm for 7 min at 4°C. The yeast cells were then resuspended in 100 μl of PBS-BSA-Ca^{2+} buffer in the presence or absence of EDTA (5 mM) with 10 μg of the appropriate collectin per ml and incubated for 1 h at 37°C. The organisms were then washed twice with 3 ml of incubation buffer (PBS-BSA-Ca^{2+}). The first layer of rabbit anticollectin antibody (40 μg/ml for anti-SP-A and anti-MBP, 1/100 for anti-SP-D, and 1/200 for anti-CL-43) was made up to 100 μl and suspended with the *C. neoformans*-collectin complex for 1 h at 4°C (the anticollectin antibodies were preincubated with the yeast cells for 30 min to remove nonspecific binding to the organism). After a repeated washing as described above, the final layer of goat anti-rabbit Ig–FITC-labelled antibody (1/200 diluted in PBS-BSA-Ca^{2+} buffer) was incubated for 1 h at 4°C. Excess FITC-labelled antibody was washed off, and the yeast cells were fixed with 1 ml of absolute methanol for 10 min at room temperature. The yeast cells were then washed once again, resuspended in PBS, and analyzed.

Agglutination assay. Acapsular B4131 or encapsulated B3501 *C. neoformans* was washed twice in PBS-BSA-Ca^{2+} buffer and centrifuged at 2,000 rpm for 7 min at 4°C. The yeast cells were then resuspended in 100 μl of buffer at 10^6 cells per well into a 96-well flat-bottom tissue culture plate (Falcon, Oxford, United Kingdom). The yeast cells were incubated at 37°C with each collectin (SP-D, SP-A, MBP, and CL-43) at a concentration of 10 μg/ml and observed by light microscopy (Zeiss, Jena, Germany).

RESULTS

Binding characteristics of pulmonary surfactant proteins SP-A and SP-D. The first contact of *C. neoformans* with the host occurs in the lungs following inhalation of acapsular or sparsely encapsulated yeast cells from the environment. Our initial experiments therefore examined the characteristics of binding of the pulmonary collectins SP-A and SP-D to a stable

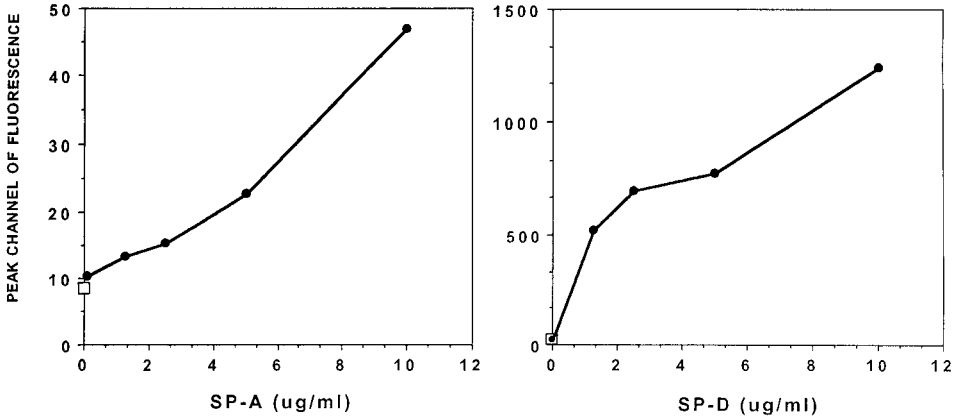

FIG. 2. Concentration dependent binding of SP-A and SP-D to acapsular *C. neoformans*. *C. neoformans* (10^6 cells) was incubated for 1 h at 37°C with various concentrations of SP-A or SP-D. Data are presented as PkChl fluorescence. The data show the results of a representative experiment of two. □, unstained control; ●, collectin plus rabbit anticollectin, goat anti-rabbit Ig–FITC-labelled antibody.

TABLE 1. Effect of EDTA on binding of SP-A and SP-D to acapsular *C. neoformans*

C. neoformans treatment group[a]	PkChl no. fluorescence	
	SP-A	SP-D
Unstained	8.4	8.4
Antibody control	17.2	10.0
Collectin	50.0	638.6
Collectin + EDTA	22.0	13.3

[a] Acapsular *C. neoformans* (10^6 cells) was incubated with either 10 μg of SP-A or SP-D per ml in 1.5 mM $CaCl_2$ either with or without 5 mM EDTA. The unstained group indicates the autofluorescence of *C. neoformans* only. The first-layer antibody was a rabbit anti-SP-A or anti-SP-D antibody; the second-layer antibody was a goat anti-rabbit Ig–FITC-labelled antibody. The collectin group was *C. neoformans* and SP-A or SP-D incubated in the presence of $CaCl_2$ followed by rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies. Collectin plus EDTA was *C. neoformans* and SP-A or SP-D in the presence of $CaCl_2$ and EDTA followed by rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies. The data are representative results of three experiments.

acapsular mutant of *C. neoformans* (B4131). Purified human SP-A and SP-D were incubated with viable yeast cells, and the bound complex was detected with rabbit anti-SP-A or anti-SP-D antibody followed by goat anti-rabbit Ig–FITC-conjugated antibody. As demonstrated by flow cytometry, 82% of the yeast cells stained positive for SP-A, whereas 97% showed binding for SP-D with calcium ion-containing buffer (Fig. 1). The interaction of both SP-A and SP-D with the organism was concentration dependent, with demonstrable binding observed at concentrations of 2.5 μg/ml for SP-A and 1.2 μg/ml for SP-D, which then increased in a dose-dependent manner (Fig. 2). Coincubation of surfactant proteins plus yeast cells in Ca^{2+} buffer supplemented with 5 mM EDTA reduced the number of positively stained yeast cells from 82 to 21% for SP-A and from 97 to 14% for SP-D (Table 1), confirming the calcium dependency of the binding event.

To assess the carbohydrate specificity of SP-A and SP-D, competition assays were performed in the presence or absence of defined saccharides. SP-A has previously been reported to have a high affinity for *N*-acetylmannosamine, and competition with this sugar at a concentration of 500 mM reduced SP-A binding by 81%, corresponding to a reduction in peak channel (PkChl) number of fluorescence intensity from 56 to 11 (Fig. 3A). *N*-acetylglucosamine also inhibited SP-A binding to *C. neoformans* but with approximately fivefold less potency than *N*-acetylmannosamine (Fig. 3A). In contrast, SP-D has a high affinity for maltose, and coincubation of this competitor at a concentration of 500 mM reduced SP-D binding by 90%, corresponding to a reduction in PkChl number of fluorescence intensity from 316 to 29 (Fig. 3B). Under these conditions, galactose was a less effective competitor, reducing binding by 33% (PkChl number reduction from 316 to 213), whereas *N*-acetylglucosamine showed only minimal competition of 13% (PkChl number reduction from 316 to 274).

Binding characteristics of serum collectins MBP and CL-43 to *C. neoformans*. We extended our initial experiments by examining the binding of serum collectin proteins MBP and CL-43 to the acapsular yeast. FACScan analysis (expressed in PkChl numbers) showed binding of purified human MBP: autofluorescence of yeast cells, 4.5; antibody control, 9; MBP, 149. Bovine CL-43 also showed binding to the acapsular yeast: autofluorescence of yeast cells, 11; antibody control, 83.5; CL-43 407 (PkChl numbers). The interaction of MBP and CL-43 with the organism was concentration dependent, with demonstrable binding observed at concentrations of 1.25 μg/ml for MBP and 2.5 μg/ml for CL-43, which then increased in a dose-dependent manner (maximal concentration tested, 10 μg/ml). The fluorescence intensity of MBP binding was reduced by 85% in the presence of 5 mM EDTA, indicating a calcium-dependent reaction (PkChl number reduction from 149 to 23 with a unimodal shift). Furthermore, binding to acapsular *C. neoformans* was also reduced by 67% in the presence of 500 mM mannose, consistent with an interaction of the lectin domain of the collectin with the yeast cell wall (PkChl number reduction from 149 to 49). Together these results suggest that in addition to the pulmonary surfactant proteins the serum collectins MBP and CL-43 also bind to the cell wall of *C. neoformans.*

Effect of capsule synthesis on binding of collectins to *C. neoformans*. The polysaccharide capsule of *C. neoformans* is an essential determinant of virulence in vivo, primarily because of the prevention of phagocytosis of the organism. The potential interaction of collectins with the capsular polysaccharides was investigated by comparing binding to the encapsulated *C. neoformans* isolate B3501 and to the acapsular mutant B4131 from which it was derived. Infection of mice by intraperitoneal in-

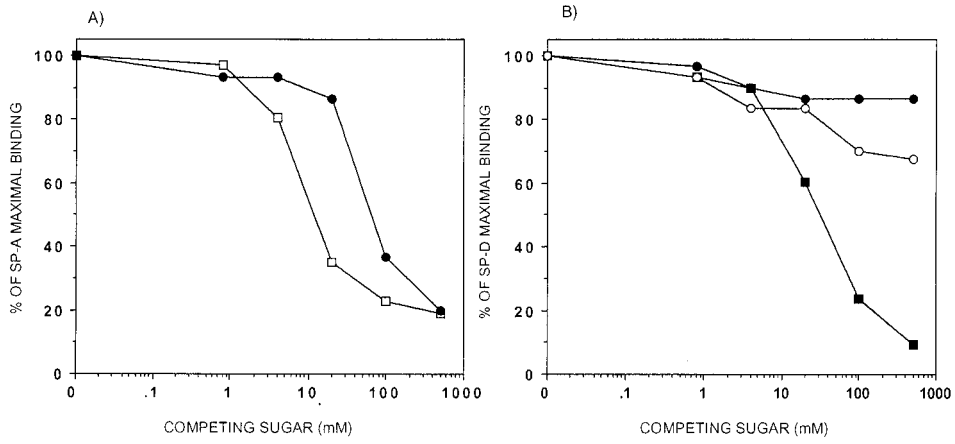

FIG. 3. Competition of SP-A and SP-D binding to acapsular *C. neoformans* by selected sugars. *C. neoformans* (10^6 cells) was incubated with 10 μg of SP-A (A) or SP-D (B) per ml and competing sugars at various concentrations. Data are presented as the percentage of maximal binding obtained from PkChl numbers of SP-A or SP-D in the absence of the competitor (PkChl number 56 or 316, respectively). The data are representative results of two experiments. ●, GlcNAc; ■, maltose; ○, galactose; □, ManNAc.

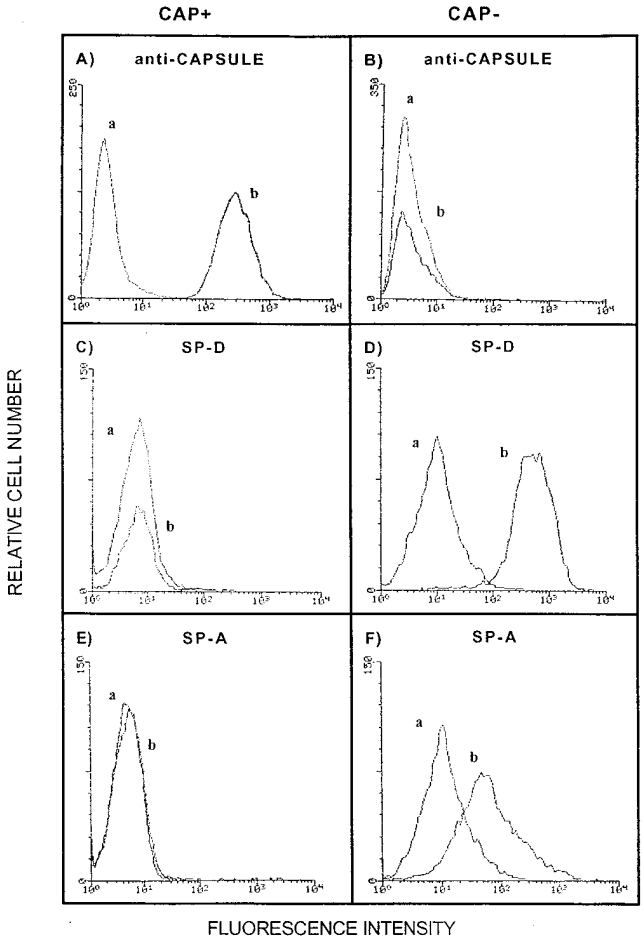

FIG. 4. Effect of capsule synthesis on binding of collectins to *C. neoformans*. *C. neoformans* B4131 (CAP^-) (B, D, and F) and B3501 (CAP^+) (A, C, and E) were stained with a rabbit anticapsule antibody followed by a goat anti-rabbit Ig–FITC-labelled antibody (A and B) or tested for binding to SP-D (C and D) or SP-A (E and F) as described in Materials and Methods. The results of FACScan analysis of 5,000 yeast cells are shown. (A and B) *C. neoformans* with normal rabbit serum plus goat anti-rabbit Ig–FITC-labelled antibody (a) and *C. neoformans* with rabbit anticapsule and goat anti-rabbit Ig–FITC-labelled antibodies (b); (C to F) *C. neoformans* plus buffer with rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies (a) and *C. neoformans* plus collectin with rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies (b).

jection with 3×10^7 B3501 cells was lethal by 32 days, whereas all mice challenged with B4131 survived, confirming the association of virulence with capsule synthesis in these two isolates. Differences in capsule expression between the two strains were then confirmed by flow cytometry with a specific anticapsule antibody. FACScan analysis of B3501 and B4131 stained with an anticapsule antibody showed that 85% of the B3501 strain stained positively compared with 3.1% for B4131 (Fig. 4A and B). Both strains were then incubated with an optimal amount of each surfactant protein, and binding was assessed by flow cytometry. At a concentration of 10 μg/ml, the encapsulated yeast showed no detectable binding of the surfactant proteins SP-A and SP-D (Fig. 4C and E) in contrast to significant responses seen in the absence of the capsule (Fig. 4D and F). The serum collectins MBP and CL-43 were also investigated, but again no binding to encapsulated organisms was observed (data not shown). The differences in the interactions of the two variants B3501 and B4131 with collectins presumably reflect different carbohydrate structures between the yeast cell wall and the polysaccharide capsule.

Binding of human SP-D causes agglutination of acapsular *C. neoformans*. The functional consequences of collectin binding to pathogens remain uncertain, but one potential response is to induce agglutination of the organism and to restrict its dissemination within the host. To study whether collectins are capable of agglutinating *C. neoformans*, 10^6 acapsular yeast cells were incubated with 10 μg of either surfactant proteins SP-A and SP-D or MBP and CL-43 per ml in calcium-containing buffer and observed by light microscopy. Within 30 min of incubation at 37°C, SP-D induced demonstrable clumping compared with yeast cells incubated in buffer alone, and complete three-dimensional agglutination occurred by 1.5 h (Fig. 5G). This complex could be disrupted by vigorous pipetting or vortexing but was reformed within 10 min at room temperature. The agglutinated yeast-surfactant protein complex was stable for up to 1 week at 4°C. In contrast, SP-A, MBP, and CL-43 were not capable of agglutinating *C. neoformans*, even with incubation for up to 24 h (Fig. 5C and E) despite demonstration of their quantitative binding by FACScan analysis. Incubation of SP-A, SP-D, or MBP with encapsulated *C. neoformans* was also performed under the same conditions as those for acapsular yeast cells, but no agglutination was observed in any group (Fig. 5D, F, and H), consistent with the absence of binding to encapsulated yeast cells. Finally, to distinguish whether the kinetics of agglutination reflected the actual rate of binding of SP-D to the organism or the rate of agglutination, a more detailed time course of SP-D binding to acapsular yeast cells was studied. Maximal binding of purified SP-D to acapsular *C. neoformans* was observed by 30 min of incubation, with 50% of binding occurring within 5 min of contact between the collectin and the organism (Fig. 6). These experiments suggest that SP-D binds rapidly to carbohydrate components of the cell wall of acapsular *C. neoformans* and, in addition, results in agglutination and immobilization of the organism in vitro.

DISCUSSION

In this study we investigated the binding of purified collectins to the fungal pathogen *C. neoformans*. We compared two variants of the yeast, a stable acapsular mutant representing a possible initial infectious form of the organism inhaled from the environment and the encapsulated parent strain representing the virulent tissue phase of the organism. Three major observations arose from these experiments. Firstly, the pulmonary surfactant proteins SP-A and SP-D as well as the serum collectins MBP and CL-43 bind to the cell wall of acapsular *C. neoformans*. Secondly, the expression of the polysaccharide capsule by virulent *C. neoformans* abolishes recognition by all collectins tested. Finally, binding of SP-D (but not other collectins) to the acapsular yeast led to stable and extensive agglutination of the organism in vitro.

C. neoformans is an important pulmonary pathogen and is believed to initiate infection in the lung primarily in its acapsular form. We therefore investigated whether the lung surfactant proteins SP-A and SP-D interact with the yeast. Both SP-A and SP-D bound specifically to *C. neoformans* at concentrations consistent with those in previous in vitro observations with other organisms (27) and within the range of concentrations measured in the host (29). Under these conditions, binding of SP-D consistently resulted in a 10- to 17-fold higher fluorescence intensity than that of SP-A (four experiments). Whether this reflects a greater affinity of SP-D for the yeast cell wall is not clear since different anticollectin antisera were used

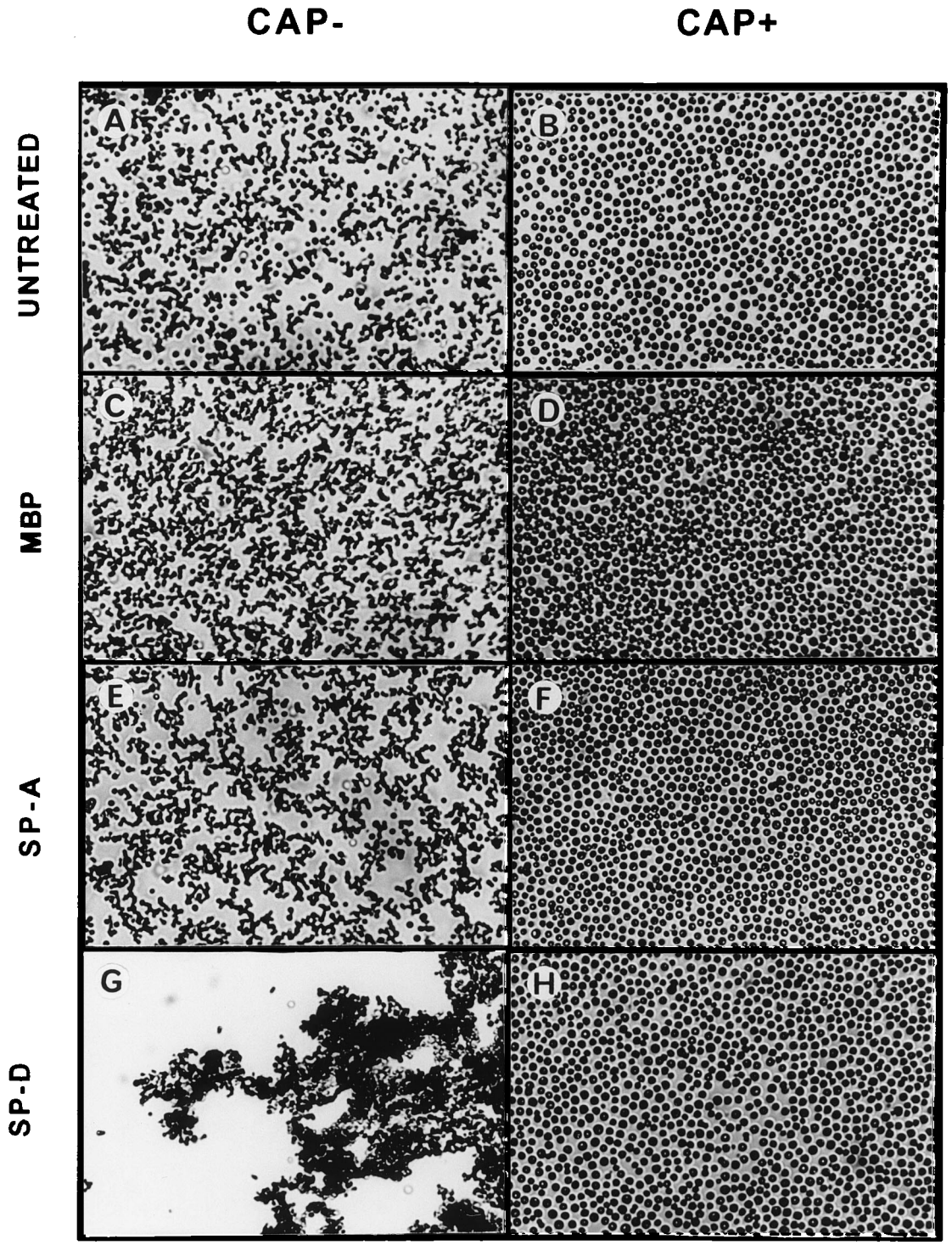

FIG. 5. Agglutination of acapsular *C. neoformans* observed by light microscopy after incubation with various collectins. *C. neoformans* (10^6 cells) was incubated with either 10 μg of SP-D, SP-A, or MBP per ml in a 1.5 mM $CaCl_2$ buffer for 60 min, and agglutination was assessed by phase-contrast microscopy (magnification, ×10). (A) Untreated acapsular *C. neoformans* (CAP^-); (B) untreated encapsulated *C. neoformans* (CAP^+); (C) CAP^- plus MBP; (D) CAP^+ plus MBP; (E) CAP^- plus SP-A; (F) CAP^+ plus SP-A; (G) CAP^- plus SP-D; (H) CAP^+ plus SP-D. The data show the results of a representative experiment of five.

for detection. Binding of both surfactant proteins was inhibited in the presence of EDTA, consistent with an interaction with the cell wall of *C. neoformans* through the C-type lectin domains present on the globular head region of the collectin. Our findings correlate with data from Zimmerman et al. (40) showing calcium-dependent binding for SP-A to mannose moieties present on *Pneumocystis carinii* and binding of SP-D to bacterial lipopolysaccharides of *Escherichia coli* (18). However, at the present time only a limited number of microorganisms have been examined for recognition by SP-A or SP-D and of

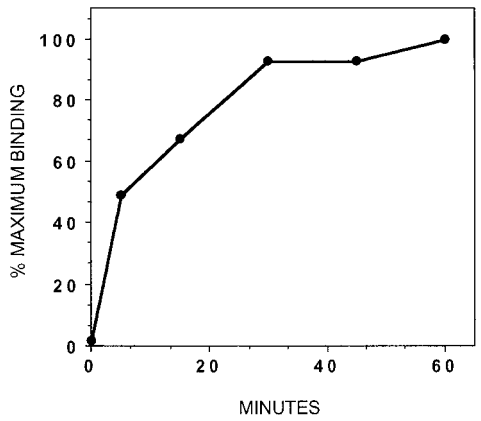

FIG. 6. Kinetics of SP-D binding to acapsular *C. neoformans*. *C. neoformans* (10^6 cells) was incubated for various times with 10 μg of SP-D per ml followed by rabbit anticollectin and goat anti-rabbit Ig–FITC-labelled antibodies. Data are presented as the percentage of maximum binding observed at 60 min of incubation (PkChl number 523.3 versus 8.66 for control yeast cells in the absence of collectin).

these only *Streptococcus pneumonia* (27), *P. carinii* (40), and influenza A virus (9) are true respiratory pathogens. The data presented here now provide evidence that both surfactant proteins SP-A and SP-D bind to another pulmonary pathogen and clinically important yeast.

Collectins can distinguish nonself carbohydrates on microorganisms from self glycoproteins via their CRDs. The structure of the CRD determines the collectin specificity, which can be demonstrated by binding competition assays using defined saccharides (13). The sugar competition data presented here showed that binding of SP-A can be effectively inhibited by *N*-acetylmannosamine and *N*-acetylglucosamine and that binding of SP-D can be inhibited by maltose and galactose. This inhibition agrees with the carbohydrate selectivity of CRD from these collectins shown by Holmskov et al. (13). Little is known about the actual molecular architecture of the cell wall of *C. neoformans*, but composition analysis has described a structure containing 86% glucose, 7.3% *N*-acetylglucosamine, and no mannan (15). In contrast, Vartivarian and colleagues (36) reported a galactoxyloprotein containing glucose and galactose (51%), mannose (10%), and xylose (3%) which is associated with the cell wall in a noncovalent manner and can be also found in culture supernatants of acapsular *C. neoformans* (30). At present, we do not know the specific carbohydrates of the yeast involved in binding of SP-A or SP-D, and we are currently attempting to identify the sugar residues of *C. neoformans* involved this interaction. It is most likely that the individual CRDs of the collectin oligomer can simultaneously bind to a combination of different carbohydrates to achieve high-affinity binding and that this combination of carbohydrates may differ for each collectin examined.

Our studies also demonstrate the binding of two serum collectins, namely MBP and CL-43, to the cell wall of *C. neoformans*. These experiments were initiated through a previous observation in our laboratory that ingestion of acapsular yeast cells by murine macrophages involved the mannose receptor and that human recombinant MBP, which possesses a similar binding specificity, also binds to the acapsular organism (3). The concentrations required for MBP and CL-43 binding presented here are consistent with other reports of serum collectin binding to pathogens (27, 35). The serum protein CL-43 has a carbohydrate specificity similar to that of MBP and structural homology to both conglutinin and SP-D (14). To our knowledge the experiments with *C. neoformans* represent the first direct demonstration of the interaction of CL-43 with a pathogen. However, limited data have been published about the opsonic activities of CL-43, and its clinical significance remains to be determined since to date it has been isolated only from bovine serum. In contrast, MBP is known to bind and opsonize bacteria and viruses which express high levels of mannose glycans, such as *Salmonella montevideo* (19, 31), human immunodeficiency virus envelope glycoprotein gp120 (5), and influenza A virus (9, 10), and its functional significance in immune defense is underlined by the fact that children with an MBP deficiency suffer from recurrent infections (32). The functional consequence of the binding of serum collectins MBP and CL-43 to the acapsular yeast is unclear, as the organism is presumably observed in the serum only in its encapsulated form. Nevertheless, immobilized MBP can increase attachment of complement-opsonized encapsulated *C. neoformans* by human phagocytes (22).

The polysaccharide capsule of *C. neoformans* is the major virulence factor so far described for the organism. Its major component, glucuronoxylomannan, consists of an α-1,3-mannose backbone with xylosyl and glucuronosyl side chains (1). In our experiments, the presence of capsule polysaccharides clearly prevented binding of collectins to the yeast. It is possible that side chain substitution of the mannose core with certain sugar residues (β-D-xylopyranosyl) may interfere with the binding of collectin globular heads. This has previously been implicated in the reduced binding rate of the complement component C3 to *C. neoformans* serotypes B and C versus serotypes A and D (37, 39). The cryptococcal capsule also interferes with other host responses such as phagocytosis via the mannose or β-glucan receptors and binding of naturally occurring anti-*C. neoformans* cell wall antibodies (17). Clearly, regardless of the molecular mechanisms involved, the data presented here demonstrate that the capsule also prevents binding of potentially opsonic collectins to the yeast cell wall, consistent with the association of virulence with capsule synthesis in vivo.

This study provides direct evidence that the collectins SP-A and SP-D as well as MBP and CL-43 bind to the fungal pathogen *C. neoformans*. We believe that the demonstration of SP-A and SP-D binding to the acapsular *C. neoformans* is of significance since both are essential constituents of the lung environment (4). In addition, we demonstrate that SP-D is also capable of agglutinating the organism in vitro. The agglutinating properties of human SP-D most likely reflect its cross-shaped structure as seen by electron microscopy, which is different from and much larger than the tulip-like structures of MBP and SP-A, allowing it to form an interlinked network with the organism (13). This phenomenon has also been reported for several gram-negative bacteria (18) and influenza A virus (9). The binding of SP-D to the yeast and subsequent agglutination may immobilize the pathogen in the lung so that alveolar macrophages can be attracted and involved in the initial control of cryptococcal infection. It has been reported that SP-A can stimulate chemotaxis of alveolar macrophages (38). Surfactant proteins have also been shown to enhance host defense mechanisms, such as increased phagocytosis of opsonized herpes simplex virus by rat alveolar macrophages in the presence of SP-A and enhancement of oxygen radical production by SP-D (33, 34). In addition, SP-A as well as crude surfactant can stimulate the production of tumor necrosis factor alpha by monocytes (8). Our laboratory has recently demonstrated that ingestion of acapsular yeast cells by macrophages triggers expression of a range of proinflammatory

cytokines including tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, interleukin 1, and macrophage inflammatory protein 1 alpha (MIP-1α) (3). Future experiments will identify whether prior opsonization of *C. neoformans* with either pulmonary or serum collectins regulates phagocytosis and subsequent macrophage activation events, including cytokine production and microbicidal activity. Finally, our observations raise the possibility that lung surfactant proteins may bind to other clinically important pathogenic fungi, including *Histoplasma*, *Blastomyces*, and *Aspergillus* species, which share common cell wall structures but do not express a polysaccharide capsule during infection.

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