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Research Article

Pneumocystis carinii is the most common cause of life-threatening pneumonia in immunocompromised patients. In the current study, surfactant protein A (SP-A), the major nonserum protein constituent of pulmonary surfactant, is demonstrated to bind P. carinii in a specific and saturable manner. SP-A is surface bound and does not appear to be internalized or degraded by the P. carinii organism. Furthermore, SP-A binding to P. carinii is time- and calcium-dependent and is competitively inhibited by mannosyl albumin. In the absence of calcium or the presence of excess mannosyl albumin, SP-A binding to P. carinii is reduced by 95 and 71%, respectively. SP-A avidly binds P. carinii with a Kd of 8 x 10(-9) M and an estimated 8.4 x 10(6) SP-A binding sites per P. carinii organism, as determined from Scatchard plots. SP-A is shown to bind P. carinii in vivo, and a putative binding site for SP-A on P. carinii is demonstrated to be the mannoserich surface membrane glycoprotein gp120. These findings suggest that P. carinii can interact with the phospholipid-rich material in the alveolar spaces by specifically binding a major protein constituent of pulmonary surfactant.

120-kD Surface Glycoprotein of *Pneumocystis carinii* Is a Ligand for Surfactant Protein A

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Abstract

***Pneumocystis carinii* is the most common cause of life-threatening pneumonia in immunocompromised patients. In the current study, surfactant protein A (SP-A), the major nonserum protein constituent of pulmonary surfactant, is demonstrated to bind *P. carinii* in a specific and saturable manner. SP-A is surface bound and does not appear to be internalized or degraded by the *P. carinii* organism. Furthermore, SP-A binding to *P. carinii* is time- and calcium-dependent and is competitively inhibited by mannosyl albumin. In the absence of calcium or the presence of excess mannosyl albumin, SP-A binding to *P. carinii* is reduced by 95 and 71%, respectively. SP-A avidly binds *P. carinii* with a K_d of 8×10^{-9} M and an estimated 8.4×10^6 SP-A binding sites per *P. carinii* organism, as determined from Scatchard plots. SP-A is shown to bind *P. carinii* in vivo, and a putative binding site for SP-A on *P. carinii* is demonstrated to be the mannose-rich surface membrane glycoprotein gp120. These findings suggest that *P. carinii* can interact with the phospholipid-rich material in the alveolar spaces by specifically binding a major protein constituent of pulmonary surfactant. (*J. Clin. Invest.* 1992. 89:143–149.) Key words: binding • lectin • pneumonia • surfactant apoprotein • AIDS**

Introduction

The most characteristic morphologic finding in *Pneumocystis carinii* pneumonia is the filling of the alveoli with an abundant network of foamy surfactant-like material that encases the *P. carinii* organisms (1–3). The accumulation of this lipid- and protein-rich material in the alveolar spaces likely contributes to the impairment of gas exchange observed in *P. carinii* pneumonia (4). However, the interaction of *P. carinii* with the components of pulmonary surfactant is poorly understood.

Surfactant protein A (SP-A),[1] a surfactant-associated protein (5), may play a role in surfactant organization and homeostasis. SP-A has been implicated in the adsorption of phospholipid to the alveolar air-liquid interface (6), the in vitro regulation of surfactant secretion (7–9) and the in vitro uptake of phospholipid by alveolar type II cells (10). In addition, SP-A is recognized as a calcium-dependent lectin with a binding specificity for immobilized mannose, fucose, glucose, and galactose (11).

The predominant surface membrane protein on *P. carinii* is a glycoprotein with an estimated molecular mass of 110–120 kD, known as *P. carinii* gp120 (12). Recent studies suggest that gp120 may serve to mediate attachment of *P. carinii* to the alveolar epithelium (13). *P. carinii* gp120 is heavily glycosylated with mannose-containing oligosaccharide chains (14) that could function as ligands for SP-A.

In this study, SP-A is demonstrated to avidly bind *P. carinii* both in vitro and in vivo. This binding is saturable, calcium dependent, and carbohydrate specific. Ligand immunoblotting of size-fractionated *P. carinii* membrane proteins demonstrate that SP-A selectively binds the carbohydrate expressed by the mannose-rich gp120. Finally, SP-A binding to *P. carinii* in vivo is verified by immunoblot analysis of *P. carinii* obtained by lung lavage using polyclonal antibody to rat SP-A.

Methods

Isolation of P. carinii. P. carinii pneumonia was induced in pathogen-free rats by immunosuppression with dexamethasone and transtracheal inoculation of *P. carinii* organisms as described by Bartlett et al. (15). *P. carinii* organisms were then harvested by lung lavage as previously described (16, 17). *P. carinii* were purified from the lavage fluid by a method adapted from Masur and Jones (18). The lavage fluid was centrifuged (600 *g* for 10 min) to pellet inflammatory and alveolar cells, and the supernatant was saved. The cell pellet was resuspended and cytopreparation smears were made using a cytologic centrifuge (Cytospin II; Shandon Southern Instruments Inc., Sewickley, PA). The cytopreparation smears were stained with Gomori methenamine silver stain (19) and Diff-Quick (Difco Laboratories, Inc., Detroit, MI) stain (20) to confirm the presence of *P. carinii* organisms. Any suspensions found to contain bacterial, fungal, or inflammatory cell contamination were discarded. The supernatant was centrifuged (2,000 *g* for 30 min) and the resulting *P. carinii* pellet was resuspended in HBSS without calcium or magnesium (Gibco Laboratories, Grand Island, NY) plus 0.6 mM EDTA. Examination of this suspension by light microscopy demonstrated *P. carinii* trophozoites to represent 97–98% of the intact cellular material present. *P. carinii* trophozoites were quantified by the method of Bartlett et al. (21).

SP-A isolation, purification, and iodination. Surfactant protein A was purified by previously described methods (22). Briefly, surfactant was obtained by lung lavage of rats 4 wk after intratracheal instillation of silica in saline. The surfactant was purified as described by Hawgood et al. (6) and delipidated by extraction with *l*-butanol. The butanol-insoluble protein precipitate was separated from the lipids by centrifugation (2,000 *g* for 30 min). The protein was suspended in 5 mM Tris/HCl buffer (pH 7.4) and dialyzed against the same buffer at 4°C for 36–48 h. The protein suspension was centrifuged (150,000 *g* for 1 h), and the supernatant was collected and then eluted over an affinity

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1. *Abbreviation used in this paper:* SP-A, surfactant protein A.

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column of mannose-Sepharose 6B (23). The SP-A bound to the column in the presence of 2 mM $CaCl_2$ was then eluted with 2 mM EDTA. The eluate was further purified and residual EDTA removed by gel filtration over a Bio-Gel A 5-m column (Bio-Rad Laboratories, Richmond, CA). Protein content was estimated using the method of Lowry et al. (24).

^{125}I-SP-A was prepared as previously described according to the method of Bolton and Hunter (25). Rat SP-A (1–2 mg) was dialyzed at 4°C against a 0.1 M sodium borate buffer (pH 8.5). A 1–1.5-ml aliquot of the SP-A suspension was added directly to an iced Bolton-Hunter Reagent vial (Amersham Corp., Arlington Heights, IL) after the benzene was removed by evaporation under a stream of nitrogen. The reaction mixture was incubated for 30 min on ice with occasional mixing. Free ^{125}I was removed by dialysis against a 5-mM Tris buffer (pH 7.4). The specific activity of the ^{125}I-SP-A was 31–54 μCi/mg protein. The purity of the SP-A and ^{125}I-SP-A obtained by these methods has previously been demonstrated (22).

Binding of ^{125}I-SP-A to P. carinii. Isolated *P. carinii* were incubated 18 h in DME (Whittaker MA Bioproducts, Walkersville, MD) supplemented with 0.6 mg/ml glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.5 μg/ml amphotericin B, 4 μg/ml gentamicin, 1% BSA, and 5 mM EGTA (pH 7.4). To prepare *P. carinii* for binding experiments, the *P. carinii* suspension was centrifuged (2,000 *g* for 15 min), the supernatant was discarded, and the pellet was washed once with HBSS containing 2 mM $CaCl_2$ supplemented with 1% BSA (HBSS$^+$) and finally resuspended in DME plus 1% BSA to a concentration of 5×10^5 *P. carinii* organisms/ml. Binding of SP-A to *P. carinii* trophozoites was quantified using a modification of the method of Proctor et al. (26). Binding assays performed to determine the specific binding of SP-A to *P. carinii* contained 5×10^4 *P. carinii* (100 μl), 60–1,500 ng ^{125}I-SP-A (10–30 μl) in sample buffer (5 mM Tris/HCl and 1% BSA, pH 7.4) in the presence and absence of 10 μg unlabeled SP-A. The reaction suspension was incubated for 30 min in an atmosphere of 90% air and 10% CO_2 at 37°C. The suspension was spun in a microfuge (13,600 *g* for 2 min). The cell pellet was washed one time with HBSS$^+$, and the pellet was added to a 12 × 75-mm tube for counting. Counts per minute present were determined using a gamma counter, and the amount of ^{125}I-SP-A bound was quantified (ng bound = CPM_{pellet}/protein specific activity). To study the effect of time, temperature, calcium, and excess carbohydrate on the binding of SP-A to *P. carinii,* binding assays were performed using 1×10^6 *P. carinii* in HBSS$^+$, 100 ng ^{125}I-SP-A, and increasing concentrations of calcium (0–2 mM), α-methyl-mannopyranoside (Sigma Chemical Co., St. Louis, MO) (0–750 mM) and mannosyl-BSA (0–1,000 μg) with a molar ratio of mannose to albumin of 26:1 (Sigma Chemical Co., St. Louis, MO). The final volume of the mixture was 160 μl. The assay was incubated as above and the amount of ^{125}I-SP-A bound was quantified (ng bound = $100 \times CPM_{pellet}/[CPM_{pellet + supernatant}]$).

The ability of anti-SP-A IgG to inhibit binding was also examined in a similar manner using 0, 1.0, and 2.5 μg of antibody. Polyclonal rabbit anti-rat SP-A IgG was raised in New Zealand white rabbits. The animals were injected with 50–100 μg of purified rat SP-A in Freund's complete adjuvant. A second booster injection of 50–100 μg of rat SP-A in Freund's incomplete adjuvant was given at 14 d. The titer of antibody was assayed 10 d after the booster injection by ELISA using purified rat SP-A as the solid phase. A repeat booster injection was given in cases where the titer was < 1:10,000. Antibody was isolated from immune rabbit serum on Protein-A Sepharose CL-4B (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) and eluted with 0.1 M acetic acid. The eluted fraction was neutralized immediately in Tris buffer and adsorbed against rat serum bound to Affigel-10 and Affigel-15 columns (Bio-Rad Laboratories). The antibody was exhaustively dialyzed against PBS and stored at −20°C. The purity and specificity of this antibody has previously been demonstrated (9).

To examine whether SP-A is surface bound or internalized, binding assays were performed as above at 37° and at 4°C, a temperature at which internalization is inhibited. A second method to determine whether ^{125}I-SP-A was surface bound used release of ^{125}I-SP-A from *P. carinii* by washing the cells with 5 mM Tris/HCl plus 5 mM EGTA (pH 7.4). Since the binding of SP-A to *P. carinii* is calcium dependent, this treatment releases surface-bound but not internalized ^{125}I-SP-A.

Finally, autoradiography and TCA precipitation of purified and bound ^{125}I-SP-A were used to study whether bound SP-A remains intact or is degraded after binding. *P. carinii* bound by ^{125}I-SP-A were solubilized in SDS-PAGE sample buffer (0.125 M Tris, 20% glycerol, 4% SDS, 0.002% bromophenol blue, and 4% 2-mercaptoethanol), and the proteins were size fractionated by SDS-PAGE. The gel was dried and exposed to Kodak X-Omat RP film for 2 h at −70°C. TCA precipitation of purified and *P. carinii*–bound ^{125}I-SP-A was done using a TCA final concentration of 26%.

Binding of SP-A to P. carinii membrane proteins. Rat *P. carinii* organisms obtained from culture (27) were solubilized in 9.5 M urea, 2% (wt/vol) NP-40, 2% ampholines (composed of 1.6% pH range 5–7 and 0.4% pH range 3–10) and 5% 2-mercaptoethanol. The *P. carinii* proteins were then electrofocused and size fractionated by two-dimensional gel chromatography as described by O'Farrell (28). To demonstrate whether SP-A binds a *P. carinii* surface protein, the protein in the two-dimensional gel was electrophoretically transferred for 30 min to an Immobilon-P PVDF transfer membrane (Millipore Corp., Bedford, MA) using Towbin buffer (29) supplemented with 0.05% SDS. The membrane was blocked with 3% BSA, 5 mM Tris, 2 mM $CaCl_2$, 0.5% Triton X-100 pH 7.4 (buffer A) for 2 h. The membrane was blotted with 20 μg/ml SP-A in buffer A for 2 h at room temperature. After this, all washes and incubations were done at 4°C. After each incubation the membrane was washed five times, 5 min/wash with buffer A. The membrane was immunoblotted with 20 μg/ml polyclonal rabbit antibody to rat SP-A for 2 h. The bound SP-A was visualized using horseradish peroxidase–conjugated goat anti–rabbit IgG antibody developed with 4-chloro-1-naphthol in diethylene glycol (Bio-Rad Laboratories).

Demonstration of in vivo binding of SP-A to P. carinii. To demonstrate whether SP-A is bound to *P. carinii* organisms in vivo, organisms were collected as before in lavage fluid containing 2 mM $CaCl_2$. Lung lavage from uninfected animals was obtained and purified in a similar fashion and used as a control. The *P. carinii* obtained from lung lavage were centrifuged (71,000 *g* for 90 min) through a 20–60% discontinuous sucrose density gradient. The cell band present at the 20–30% (0.58–0.88 M) sucrose interface was collected and solubilized in SDS-PAGE sample buffer. The *P. carinii* and the corresponding control fractions were subjected to SDS-PAGE, transferred to nitrocellulose, and then immunoblotted with rabbit polyclonal antibody to rat SP-A. The presence of SP-A was identified using horseradish peroxidase–conjugated goat anti–rabbit IgG antibody directed against the rabbit polyclonal anti-SP-A antibody.

Statistical analysis. Values are expressed as means±SEM. Differences between control and experimental data groups were compared using the one-tailed *t* test or ANOVA with paired comparisons performed using Fisher's LSD method. Statistical significance was accepted for $P < 0.01$.

Results

^{125}I-SP-A binding to P. carinii trophozoites. SP-A binds *P. carinii* in vitro in a time-dependent manner and achieves apparent equilibrium by 30 min (Fig. 1). At 5 min 34.45±0.62 ng ^{125}I-SP-A/1×10^6 organisms is bound, whereas at the observed times of 30 min and beyond, mean binding of ^{125}I-SP-A plateaus at 52.62±0.56 ng/1×10^6 organisms. Further binding experiments were conducted at 30 min, a time when equilibrium of SP-A binding was established.

The calcium requirement for optimal binding of SP-A to *P. carinii* was examined by repeating the SP-A binding assays in the presence of increasing concentrations of extracellular calcium (Fig. 2). In the absence of calcium only 2.85±0.27 ng of ^{125}I-SP-A is bound, whereas in the presence of 2 mM calcium

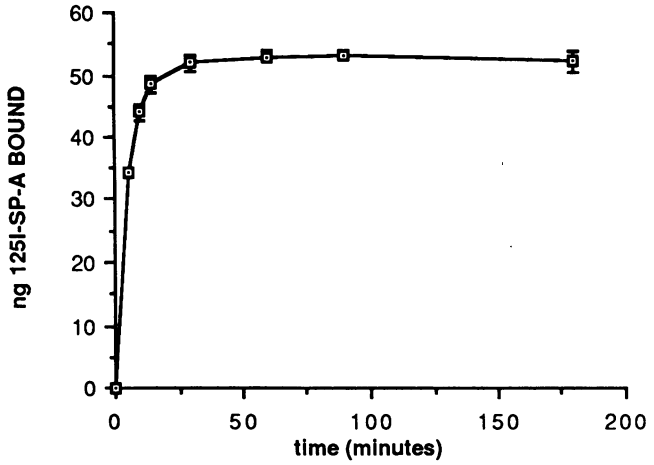

Figure 1. ^{125}I-SP-A binds *P. carinii* in a time-dependent fashion. Binding assays were performed using 1×10^6 *P. carinii* incubated with 100 ng ^{125}I-SP-A in HBSS, 2 mM calcium, and 1% BSA for the indicated times. Binding equilibrium is evident at 30 min, with 51.92±1.33 ng ^{125}I-SP-A bound/1×10^6 organisms. Values are means±SEM of three experiments performed in duplicate.

59.96±3.05 ng SP-A is bound. Binding appears to plateau beyond calcium concentrations in excess of 1 mM calcium. This is in agreement with previous studies, demonstrating the calcium requirement of SP-A ligand binding (11). Therefore, extracellular calcium appears to also be required for the optimal binding of SP-A to *P. carinii.*

The specificity and ligand concentration dependence of ^{125}I-SP-A binding to *P. carinii* was demonstrated using increasing amounts of ^{125}I-SP-A (60–1,500 ng) in the presence and absence of 10 μg excess unlabeled SP-A (Fig. 3 *A*). Addition of increasing amounts of ^{125}I-SP-A to the binding assay resulted in an increase in both total and nonspecific binding, with saturation of specific binding evident at concentrations in excess of 1,000 ng ^{125}I-SP-A added. Scatchard plots (30) using the specific binding data are linear (r = 0.99), indicating a homogenous population of binding sites for SP-A on *P. carinii* (Fig. 3 *B*). The binding dissociation constant (K_d) is 8×10^{-9} M, with each *P. carinii* trophozoite estimated to have 8.4×10^6 binding sites for SP-A, using an estimated molecular mass for SP-A of 750 kD (31, 32).

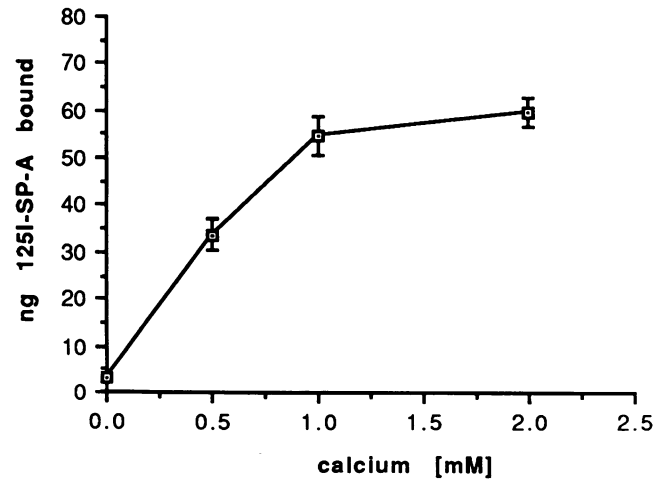

Figure 2. Calcium is required for ^{125}I-SP-A to bind *P. carinii.* Binding assays were performed as in Fig. 1, using increasing amounts of extracellular calcium. Maximal binding reveals a plateau at calcium concentrations above 1 mM. Values are means±SEM of three experiments performed in triplicate.

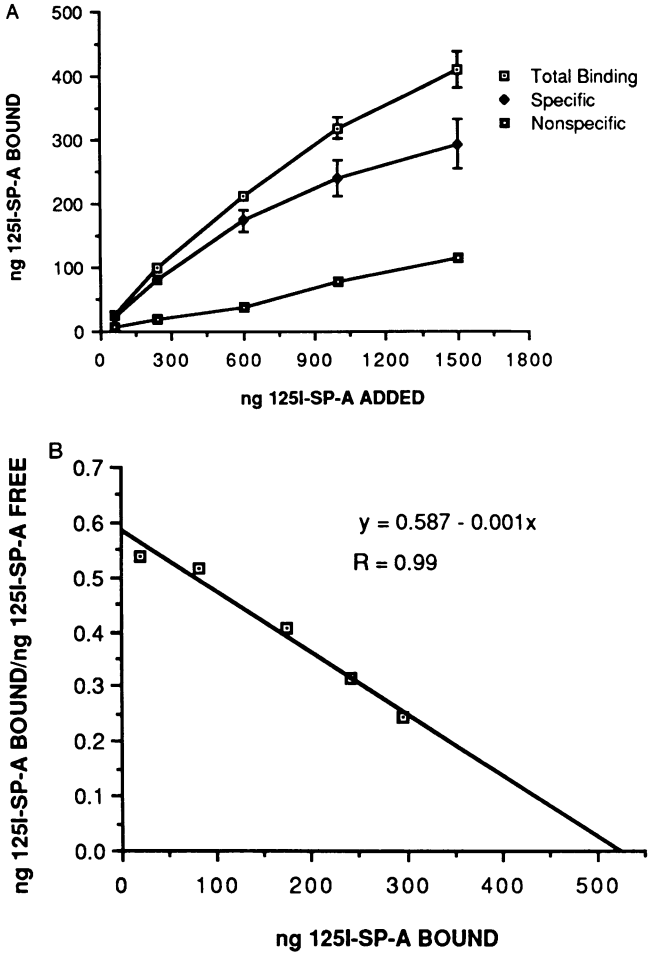

Figure 3. Saturation and specific binding of ^{125}I-SP-A to *P. carinii.* Binding assays were performed using 5×10^4 *P. carinii,* 60–1,500 ng ^{125}I-SP-A with and without 10 μg excess unlabeled SP-A. (*A*) Saturation of specific SP-A binding to *P. carinii* demonstrated in the presence of > 1,000 ng ^{125}I-SP-A added. Values are means±SEM of three experiments performed in triplicate. (*B*) Scatchard plot of specific binding data. The slope of the line represents a K_d of 8×10^{-9} M for the binding interaction of SP-A and *P. carinii.* The number of SP-A binding sites per organism was calculated from the x-intercept to be 8.4×10^6.

P. carinii binding of SP-A occurs at the cell surface and does not appear to require active internalization of the SP-A oligomer. Binding of SP-A to *P. carinii* occurs both at 37° and at 4°C, a temperature at which membrane vesicle internalization is inhibited (33) (Fig. 4 *A*). Binding is slightly greater at 4°C (71.17±2.31 ng) compared with 37°C (50.03±2.79 ng). Surface binding is further supported by demonstrating that 92% of ^{125}I-SP-A bound at 37°C can be removed from *P. carinii* by washing the organisms with 5 mM EGTA (Fig. 4 *B*).

Autoradiography of ^{125}I-SP-A bound to *P. carinii* at 37°C reveals that SP-A is not degraded. No SP-A fragments are found upon examination of an autoradiogram prepared from an SDS-PAGE of bound ^{125}I-SP-A when compared with puri-

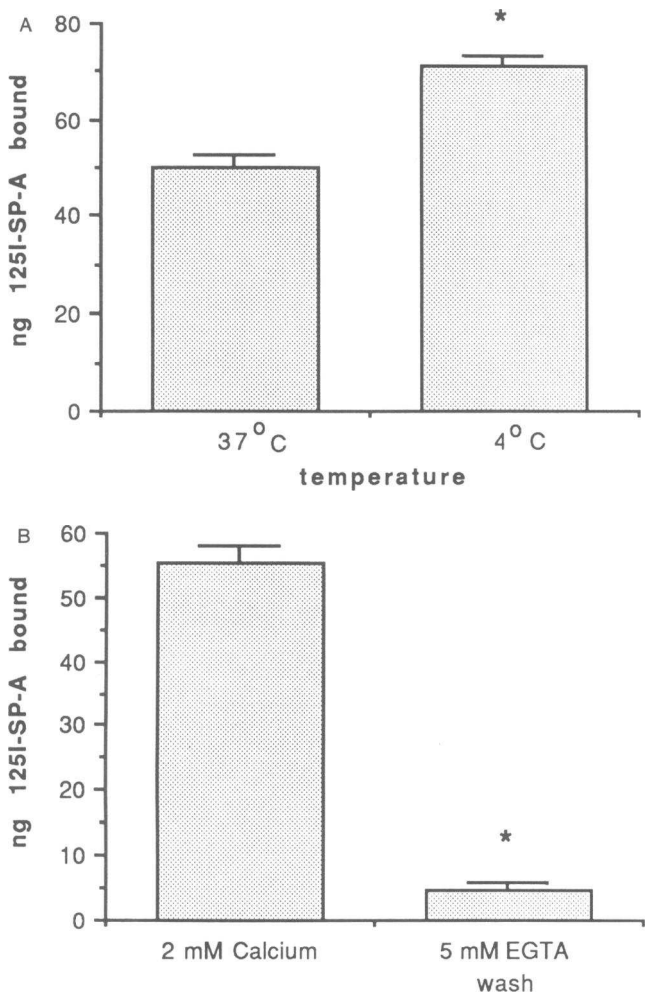

Figure 4. SP-A is bound to the cell surface of *P. carinii.* (*A*) Binding assays were performed using 100 ng ^{125}I-SP-A incubated with 1×10^6 *P. carinii* for 30 min in HBSS, 2 mM calcium, and 1% BSA at 37°C and 4°C. Binding is clearly evident at 4°C, a temperature at which receptor internalization inhibited. Values are means±SEM of three experiments performed in triplicate (*$P < 0.01$). (*B*) ^{125}I-SP-A bound to *P. carinii* at 37°C is nearly completely reversed by washing the organisms in 5 mM EGTA. Values are means±SEM of three experiments performed in triplicate (*$P < 0.01$).

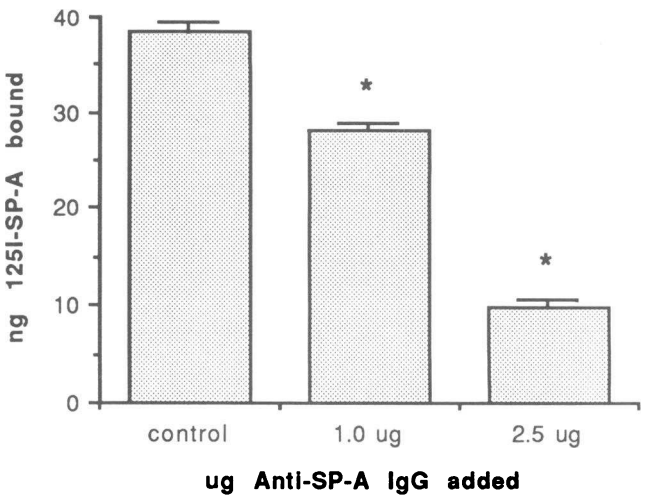

Figure 5. Polyclonal anti-SP-A IgG inhibits SP-A binding to *P. carinii.* ^{125}I-SP-A binding assays were performed in the absence and presence of polyclonal anti-SP-A IgG. SP-A binding is inhibited by 75% using 2.5 μg of antibody. Values are means±SEM of three experiments performed in triplicate (*$P < 0.01$).

fied ^{125}I-SP-A (data not shown). Furthermore, there is no difference between the TCA precipitability of purified ^{125}I-SP-A compared with the ^{125}I-SP-A incubated with *P. carinii,* 94.1 vs. 94.6%, respectively ($P > 0.50$). The failure to show a disparity between these two groups provides evidence supporting the absence of formation of small ^{125}I-SP-A peptide degradation fragments by the *P. carinii* organism.

SP-A binding to *P. carinii* could also be inhibited by polyclonal anti-SP-A IgG (Fig. 5). In the absence of antibody 38.40±3.22 ng ^{125}I-SP-A is bound, whereas in the presence of 2.5 μg anti-SP-A IgG binding is reduced to 9.77±2.05 ng.

To determine whether SP-A recognized carbohydrates expressed by *P. carinii* surface membrane glycoproteins, ^{125}I-SP-A binding experiments were conducted in the presence and absence of excess carbohydrate. Because *P. carinii* expresses high mannose–type oligosaccharides on its surface (14) and the specificity of SP-A for mannose has been well described (11), α-methyl-D-mannopyranoside and mannosyl-BSA were used to determine whether SP-A binding to *P. carinii* exhibited a specificity for carbohydrate. Mannosyl-BSA effectively inhibits ^{125}I-SP-A binding to *P. carinii* (Fig. 6 *A*). SP-A binding can be inhibited by 71% in the presence of 2.42 mM mannose when mannose is presented as the multivalent glycoprotein mannosyl-BSA, whereas the monosaccharide α-methyl-D-mannopyranoside (Fig. 6 *B*) requires concentrations as high as 500 mM to achieve a similar degree of inhibition (73%). Other monosaccharides known to be ligands for SP-A, such as *N*-acetyl-D-glucosamine also inhibit SP-A binding to *P. carinii* at high concentrations (data not shown). However, there is a limitation to the use of monosaccharides in studying SP-A binding, because the carbohydrate specificity of SP-A decreases as the monosaccharide concentration increases. The ability of low mannose concentrations, in the form of mannosyl-BSA, to inhibit binding of SP-A to *P. carinii* suggests that a glycoprotein expressed by *P. carinii* is a possible ligand for SP-A.

SP-A binding to P. carinii membrane proteins. To demonstrate the SP-A target binding site on *P. carinii,* SP-A binding to *P. carinii* membrane proteins was examined by ligand immunoblot analysis. Whole *P. carinii* proteins were resolved by two-dimensional gel chromatography and stained with Coomassie brilliant blue R-250 (Fig. 7 *A*). Two-dimensional chromatography of *P. carinii* proteins reveal a 116–120-kD basic protein, representing the majority of *P. carinii* surface protein (12) known as gp120.

The immunoreactivity for SP-A present on the ligand immunoblot analysis (Fig. 7 *B*) demonstrates that SP-A binds the *P. carinii* 120-kD surface glycoprotein, as well as a smaller protein with an estimated mass of 80 kD. There is no immunoreactivity for SP-A present when immunoblots are performed with omission of the initial SP-A incubation (data not shown). Ligand immunoblots of *P. carinii* gp120 treated with 50 mM sodium metaperiodate (34), express no immunoreactivity for SP-A (data not shown), demonstrating that oxidation of the carbohydrate residues of gp120 destroys the epitope required for SP-A binding. The inhibition of SP-A binding to whole *P. carinii* organisms with mannosyl-BSA and the periodate sensi-

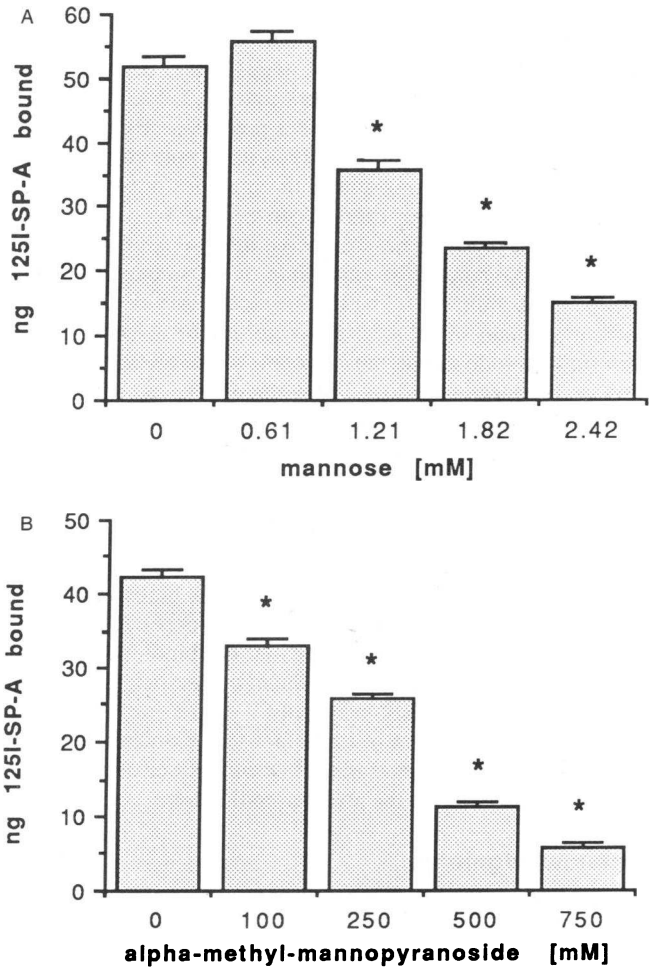

Figure 6. ^{125}I-SP-A binding to *P. carinii* is inhibited by excess monovalent and multivalent carbohydrates. (*A*) Binding assays were performed in the presence of increasing amounts of the multivalent ligand mannosyl-BSA (0–1,000 μg), resulting in an increase in monosaccharide concentration (0–2.42 mM). In the presence of 2.42 mM mannose (1 mg mannosyl-BSA), 71% of ^{125}I-SP-A binding is inhibited. There is no inhibition of ^{125}I-SP-A binding observed when identical concentrations of the carrier protein, BSA, are used (data not shown). (*B*) ^{125}I-SP-A binding assays were performed in the presence of increasing concentrations of the monosaccharide α-methyl-D-mannopyranoside. At the highest concentration tested ^{125}I-SP-A binding is inhibited by 86%. Values are means±SEM of three experiments performed in triplicate (*$P < 0.01$).

tivity of SP-A binding to *P. carinii* membrane proteins provide strong evidence that SP-A recognizes oligosaccharide moieties of gp120.

In vivo demonstration of SP-A binding to P. carinii. Because SP-A binds *P. carinii* in vitro, the pivotal issue is whether similar binding occurs in vivo within the alveolar milieu. In vivo SP-A binding to *P. carinii* was demonstrated by use of immunoblot analysis of *P. carinii* membrane proteins isolated and purified as described above. The immunoblot (Fig. 8) using polyclonal rabbit antibody to rat SP-A demonstrates the specificity of the antibody to purified SP-A (lane *3*). Lanes *1* and *4* represent identical immunoreactivity for SP-A found to be present in the isolate of *P. carinii* (lane *1*) and in *P. carinii* purified from discontinuous sucrose density gradients (lane *4*). There is no immunoreactivity for SP-A evident in the isolate obtained from pathogen-free rats (lane *2*). The absence of immunoreactivity in lane *2* is further evidence that the methods of *P. carinii* isolation from lung lavage does not also co-purify free host SP-A with the *P. carinii* organisms. This experiment provides direct evidence for the in vivo binding of SP-A to *P. carinii.*

Discussion

P. carinii organisms have previously been demonstrated to avidly bind alveolar surfactant (35); however, the mechanism of adherence between surfactant and *P. carinii* has not been defined. This study indicates that SP-A, the major protein constituent of surfactant, binds *P. carinii* in a specific and saturable fashion. Scatchard plots estimate the number of SP-A binding sites per *P. carinii* organism to be ~ 8.4×10^6. The linearity of the plots suggest that a homogeneous group of binding sites exist, with a similar affinity for SP-A.

SP-A exists in vivo as a large multivalent oligomeric protein composed of ~ 18 subunits, similar to that of complement component C1q (36). Analysis of the deduced primary amino

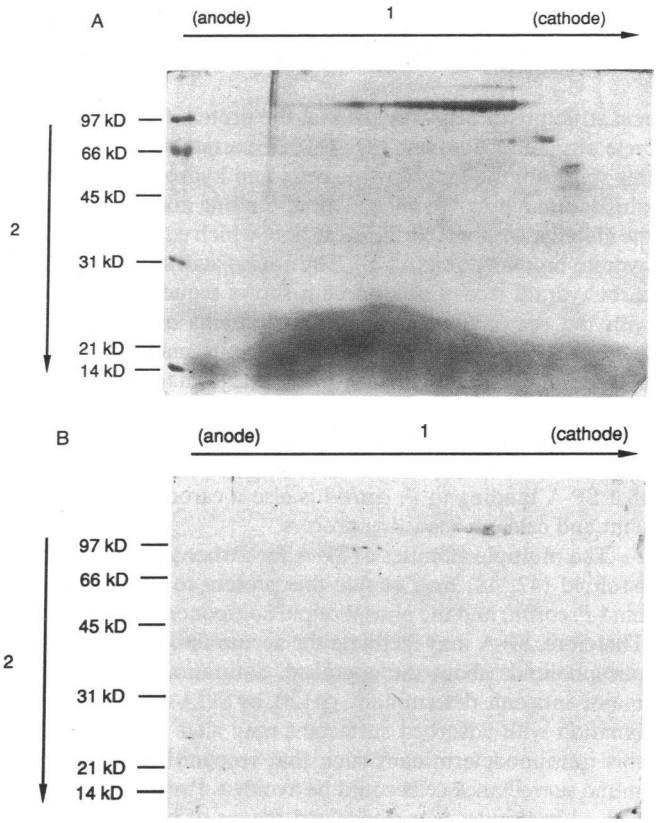

Figure 7. SP-A binds *P. carinii* gp120. (*A*) Two-dimensional gel chromatography of *P. carinii* proteins. *P. carinii* organisms were electrofocused (1, first dimension) using ampholines with a pH range of 3 to 10 and then size fractionated by SDS-PAGE (2, second dimension) and stained with Coomassie brilliant blue. The predominate band seen represents *P. carinii* gp120. (*B*) Ligand immunoblot of SP-A binding whole *P. carinii* proteins. SP-A binds almost exclusively to the protein band corresponding to the mannose-rich cell surface glycoprotein gp120. In addition there is a small amount of immunoreactivity for SP-A present in the 80-kD area representing SP-A binding to a lesser expressed *P. carinii* glycoprotein.

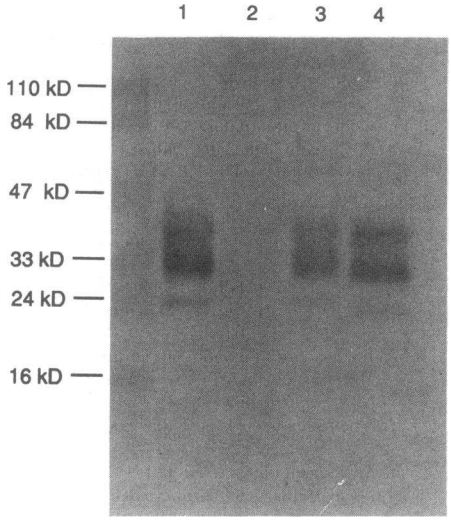

Figure 8. P. carinii binds SP-A in vivo. *P. carinii* membrane proteins were prepared and immunoblotted using polyclonal antibody to rat SP-A as described in the methods. Immunoreactivity for SP-A is demonstrated in the same 26–38 kD range in the *P. carinii* membrane protein preparations (lanes *1* and *4*) as is visible in the purified SP-A control lane (lane *3*). No immunoreactivity for SP-A is evident in lane *2*, representing alveolar lavage proteins prepared from pathogen-free rats in the same fashion as the *P. carinii* proteins.

acid structure of SP-A reveals that the protein has several discrete structural domains (37–41). Of particular relevance to this study are the amphipathic helix and hydrophobic region, which form a putative phospholipid-binding domain (42), and the globular carboxyl terminus region, which contains a carbohydrate-binding domain (43). The amino acid sequence of the carbohydrate recognition domain shows sequence homology with the carbohydrate-recognition domains of other C-type lectins, specifically the soluble serum lectin, mannose-binding protein, and the hepatic and macrophage mannose receptor (43–45). SP-A has previously been shown to require calcium for binding to immobilized saccharides or to its receptor on isolated alveolar type II cells (22, 46, 47). These data suggest that SP-A binding to *P. carinii* is also a carbohydrate-dependent and calcium-requiring process.

The multiple affinities of SP-A for carbohydrate and phospholipid (42, 48) may enable this protein to simultaneously bind *P. carinii* and the phospholipid components of surfactant. Therefore, SP-A may facilitate the accumulation of surfactant phospholipids about the organism. Saturation of *P. carinii's* major antigenic determinant, gp120, by SP-A alone or in combination with adsorbed surfactant may alter the structure of this immunodeterminant such that recognition by host immune surveillance cells could be avoided. Parasite acquisition of host molecules is a recognized means of host immune response avoidance (49). The interaction of *P. carinii* with SP-A could potentially result in the combined disruption of SP-A's regulatory role of alveolar phospholipid homeostasis and the disorganization of the alveolar phospholipid monolayer, in part explaining the alveolar filling process and hypoxia seen in *P. carinii* pneumonia (4).

The predominant antigenic determinant on the *P. carinii* cell membrane is the mannose-rich membrane glycoprotein gp120 (34, 50). In this study, SP-A is demonstrated to bind *P. carinii* gp120, supporting the hypothesis that gp120 is a putative binding site on *P. carinii* for SP-A. The ability of mannosyl-BSA to inhibit binding of SP-A to gp120 and the periodate sensitivity of SP-A binding suggests that the SP-A binding is dependent on intact oligosaccharides present on the gp120 ligand. Furthermore, immunoblot analysis of isolated *P. carinii* membrane proteins demonstrates the presence of SP-A, indicating that SP-A is adherent to *P. carinii* in vivo.

P. carinii gp120 has been demonstrated to play a potential role in the mechanism of adherence of the organism to the alveolar epithelium (13). The binding of the carbohydrate recognition domain of SP-A to *P. carinii* in the alveolar lining fluid may provide the organism access to the alveolar epithelium via fibronectin-mediated attachment between SP-A's free collagen-like domain and the alveolar epithelium. This is suggested by fibronectin's ability to bind the collagenous domain of the structurally homologous protein complement C1q (51) and fibronectin's ability to mediate attachment of *P. carinii* to the alveolar epithelium (15).

In summary, this study provides direct evidence that SP-A, the major protein constituent of surfactant, binds *P. carinii*. This binding is specific and saturable, time dependent, carbohydrate specific, and calcium requiring. The binding site on the *P. carinii* organism appears to be the mannose-rich membrane glycoprotein gp120. The binding of SP-A to *P. carinii* in vivo may enhance adsorption of surfactant to the organism, possibly impede host defenses against *P. carinii*, as well as potentiate *P. carinii* adherence to the alveolar epithelium.

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