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Research Article

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Pneumocystis carinii Attachment to Cultured Lung Cells by Pneumocystis gp120, a Fibronectin Binding Protein

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Abstract

Pneumocystis carinii **is an extracellular organism which is thought to require attachment to alveolar epithelial cells for its growth and replication in humans. Fibronectin (Fn) binding to *P. carinii* is essential for optimal *P. carinii* attachment. This study demonstrates that gp120, a 110–120-kD membrane glycoprotein on *P. carinii*, mediates attachment of the organism to cultured lung cells and is the site of Fn binding to *P. carinii*. A ^{51}Cr-labeled *P. carinii* binding assay was used to quantify attachment of the organism to the alveolar epithelial cell line A549. Addition of free gp120, purified from whole *P. carinii* organisms, caused a significant decrease in attachment of *P. carinii* to A549 cells from 44.2±5.5% to 22.4±4.2% ($P < 0.01$). Preincubation of the *P. carinii* organisms with a polyclonal antibody to gp120 also resulted in a marked decrease in *P. carinii* attachment to A549 cells from 46.8%±5.2% to 21.3±4.8% ($P < 0.01$). Furthermore, addition of free gp120 to *P. carinii* organisms caused a significant reduction in specific binding of ^{125}I-Fn to *P. carinii* (from 83.3±8.5 ng to 47.1±5.9 ng, $P < 0.01$). Similarly, anti-gp120 antibody decreased specific Fn binding to *P. carinii* from 74.3±8.4 ng to 25.5±5.3 ng ($P < 0.001$). Solubilized *P. carinii* organisms separated by gel electrophoresis and blotted with ^{125}I-Fn demonstrated specific binding of the ^{125}I-Fn to gp120. In addition, a specific anti-β_1-integrin antiserum reacted with gp120 by Western blot, suggesting structural homology between gp120 and the β-subunit of integrins. Thus, the data suggest that the *P. carinii* membrane glycoprotein gp120 functions as a Fn binding protein and is required for optimal *P. carinii* attachment to alveolar epithelial cells. (*J. Clin. Invest.* 1991. 88:403–407.) Key words: integrin • pneumonia • AIDS • immunocompromised host**

Introduction

Pneumocystis carinii pneumonia is a major cause of morbidity and mortality in immunosuppressed patients (1, 2). Current concepts suggest *P. carinii* is exclusively an extracellular parasite dependent upon attachment to alveolar cells for growth and replication of the organism (3, 4). Ultrastructural studies indicate the organism attaches to host cells by a process involving apposition but not fusion of *P. carinii* and host cell membranes (3–7). *P. carinii* organisms have been noted to bind preferentially to type I alveolar epithelial cells (3, 5–7), although attachment to type II cells has been occasionally detected (5).

Recent investigations into the cellular components of *P. carinii* have demonstrated the presence of a *P. carinii*–specific 110–120-kD surface glycoprotein (8–12), termed gp120 by Radding et al. (11). *P. carinii* gp120 is a highly glycosylated molecule containing mannose and *n*-acetyl-glucosamine residues (9, 12). The large bandwidth of gp120 observed on gel electrophoresis has been attributed to differential glycosylation of the protein component of the antigen (10). The exact structure and protein sequence of this molecule (gp120) have yet to be determined. The specific role of gp120 in the pathogenesis of *P. carinii* infection is also unknown.

This study examined the hypothesis that gp120 on the surface of *P. carinii* mediates attachment of the organism to alveolar epithelial cells. Previous work from our laboratory (13) has demonstrated that the cell-adhesive glycoprotein fibronectin (Fn)[1] binds to *P. carinii* organisms and is necessary for the optimal attachment of *P. carinii* to the alveolar epithelial cell line, A549. The results of this study indicate that gp120 is the site of Fn binding on *P. carinii* and that gp120, at least in part, is responsible for the attachment of the organism to the alveolar epithelium.

Methods

Isolation of Pneumocystis 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. (14). *P. carinii* trophozoites were harvested 4–6 wk after inoculation when the rats were moribund with *P. carinii* pneumonia. Rats were killed by intraperitoneal injection of T-61 Euthanasia Solution (Taylor Pharmacal Co., Decatur, IL), and the trachea was cannulated following a mid-line neck incision. The lungs were lavaged × 6 with 8-ml aliquots of HBSS plus 0.6 mM EDTA, penicillin 100 U/ml, streptomycin 100 μg/ml, gentamicin 4 μg/ml, and amphotericin B 0.5 μg/ml (lavage solution). Approximately 40–45 ml of lavage fluid was obtained from each rat.

P. carinii were purified by a method adapted from Masur (15). The lavage fluid was centrifuged (400 *g* × 10 min) to pellet inflammatory and alveolar cells, and the supernatant was saved. The pellet was resuspended in lavage solution to prepare cytopreparation smears (Cytospin II; Shandon Southern Instruments Inc., Sewickley, PA). The presence of *P. carinii* cysts and trophozoites was verified using Gomori methenamine silver stain (16) and Diff-Quik stain (Difco Laboratories Detroit, MI) (17). To obtain highly purified populations of *P. carinii* trophozoites, the supernatants were centrifuged (1,800 *g* × 30 min), resuspended in 1 ml of lavage solution and trophozoites quantified by the method of Bartlett et al. (18). Examination of the trophozoite suspen-

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1. *Abbreviations used in this paper:* Fn, fibronectin; TBS, Tris buffered saline.

sion showed that *P. carinii* organisms represented 97–99% of intact cellular material in the suspension. Any samples containing bacterial, fungal, or inflammatory cell contamination were discarded.

Purification of P. carinii gp120. *P. carinii* gp120 was purified by two separate methods. In the first technique, purified *P. carinii* trophozoite preparations were solubilized in a reducing SDS-PAGE sample buffer (0.125 M Tris, 4% SDS, 0.002% bromphenol blue, 20% glycerol, and 4% 2-mercaptoethanol). *P. carinii* antigens were separated by SDS-PAGE (Mini Protean II; Bio-Rad Laboratories, Richmond, CA) according to the method of Laemmli (19). To obtain a purified sample of the *P. carinii* membrane glycoprotein gp120, bands between 110 and 120 kD were cut from the gels and the protein was electroeluted (model 422 Electroeluter; Bio-Rad) from the gel. A sample of the freshly eluted *P. carinii* gp120 was placed in SDS-PAGE sample buffer and gels were repeated running the gp120 alongside the whole *P. carinii* preparations to verify the purity of the preparation. The second method for purification of *P. carinii* gp120 was performed by separation of solubilized *P. carinii* antigens by HPLC on a sizing column (TSK 3000 SW; Beckman Instruments, San Ramon, CA). Fractions corresponding to gp120 were collected and their purity verified by two-dimensional gel chromatography as described by O'Farrell (20). To prepare gp120 for use in cell culture experiments, the gp120 solutions were concentrated and transferred to HBSS using a centrifugal concentrator (Centricon 30; Amicon Corp., Danvers, MA).

Development of polyclonal anti-gp120 antibodies. Polyclonal antibodies to *P. carinii* gp120 were raised in New Zealand White rabbits (Johnson Rabbit Ranch, Mooresville, IN) by repeated intramuscular injection of eluted gp120. After the third inoculation, serum collected from one of the rabbits reacted with gp120 at a titer of 1:10,000 by Western blot, and demonstrated no cross-reactivity with normal rat lung antigens. Serum from this animal was used for all subsequent experiments.

P. carinii attachment assay. As previously described (13, 21), *P. carinii* attachment to an alveolar epithelial cell line was quantified using ^{51}Cr-labeled *P. carinii* organisms. Freshly isolated *P. carinii* trophozoites were incubated for 18 h in 2 ml of DME containing 0.5 ml of FCS and 50 μCi of ^{51}Cr sodium chromate (New England Nuclear, Boston, MA). After incubation the ^{51}Cr-labeled *P. carinii* suspension was centrifuged (1,400 $g \times 15$ min), the supernatant discarded, and the pellet resuspended in DME. The *P. carinii* suspension was washed $\times 4$ to remove unincorporated ^{51}Cr and resuspended in DME at a concentration of 20×10^6/ml.

An alveolar epithelial cell line, A549 (CCL #185; American Type Culture Collection, Rockville, MD), was used as the target cell population for *P. carinii* adherence studies. A549 cells were grown to confluency in DME + 10% FCS on 24-well tissue culture dishes. Before the attachment assay, the A549 cells were washed $\times 2$ with 1 ml of DME to remove serum. 2×10^6 ^{51}Cr-labeled trophozoites were added to each well of A549 cells and incubated at 37°C for 4 h. After the incubation, the media that contained nonadherent trophozoites were removed and saved. The cell monolayer, containing bound trophozoites, was disrupted using 10% Triton X-100 (Sigma Chemical Co., St. Louis, MO) and saved. ^{51}Cr-labeled *P. carinii* organisms were quantified in each fraction (5500 gamma counter; Beckman Instruments, Inc., Berkeley, CA) and percent attachment expressed as follows: % attachment = $(A/A + B) \times 100$, where A = ^{51}Cr-labeled *P. carinii* bound to the A549 monolayer and B = ^{51}Cr-labeled *P. carinii* free in the media.

To determine the effect of soluble gp120 on *P. carinii* attachment, the assay was performed as above with the addition of 0.5, 5, 50, 500, or 5,000 ng/ml of purified gp120. Control experiments using electroeluted albumin purified in a similar manner were also performed. To quantify the effect of the anti-gp120 antibody on attachment, ^{51}Cr-labeled *P. carinii* were preincubated with the anti-gp120 for 1 h and then added to the A549 cell monolayer. Control experiments were conducted using pooled rabbit serum and polyclonal rabbit IgG.

^{125}I-Fn binding to P. carinii. *P. carinii* trophozoites were incubated for 18 h in DME supplemented with glutamine 0.6 mg/ml, penicillin 100 U/ml, streptomycin 100 μg/ml, amphotericin B 0.5 μg/ml, gentamicin 4 μg/ml, and 0.6 mM EDTA. ^{125}I-Fn (ICN Pharmaceuticals, Inc., Irvine, CA) binding to *P. carinii* was quantified as previously described (13). Following the 18-h incubation, the *P. carinii* suspension was centrifuged (1,600 $g \times 15$ min), the supernatant discarded, and the *P. carinii*–rich pellet was resuspended in DME at 20×10^6/ml. Reaction mixtures containing 4×10^6 *P. carinii*, 3 μg ^{125}I-Fn, and sample buffer (0.02 M Hepes and 1 mg/ml BSA in 0.15 M NaCl at pH 7.4) to a total vol of 1 ml were incubated for 1 h. Mixtures were centrifuged (3,000 $g \times 2$ min) to pellet trophozoites, the supernatant was removed and saved, and the pellet was resuspended in 0.5 ml of sample buffer. Following a second centrifugation, the supernatant was again saved and added to the first supernatant. ^{125}I-Fn in the pellet (bound) and in the supernatant (free) were quantified (5500 gamma-counter; Beckman). Specific binding was determined by addition of 125 μg of unlabeled Fn (Sigma Chemical Co.) to the reaction mixture. Bound ^{125}I-Fn was calculated as follows: Bound ^{125}I-Fn = $(DPM_{pellet}/DPM_{pellet} + DPM_{supernatant}) \times$ ^{125}I-Fn added.

The effect of soluble gp120 on ^{125}I-Fn binding was determined by addition of 0.5, 5, 50, 500, or 5,000 ng of soluble gp120 to the *P. carinii* suspensions containing ^{125}I-Fn and quantifying binding as above. Similarly, the effect of increasing concentrations of the polyclonal anti-gp120 antibody on ^{125}I-Fn binding to *P. carinii* was quantified. Control experiments were again conducted using pooled rabbit serum and polyclonal rabbit IgG.

Binding of ^{125}I-Fn and anti-β_1-integrin to gp120. Purified *P. carinii* gp120 was separated by SDS-PAGE as previously described. The gp120 was then transferred to a nitrocellulose membrane (Mini Transphor; Hoefer Scientific Instruments, San Francisco, CA) by the method of Towbin et al. (22). The nitrocellulose membrane was blocked by incubation for 2 h in 5% dried milk in Tris buffered saline (TBS). The nitrocellulose membrane was then blotted with 3 μg of ^{125}I-Fn in TBS plus 0.5% albumin for 2 h. After extensive washing of the nitrocellulose membrane with TBS to remove nonadherent ^{125}I-Fn, the membrane was dried and exposed to Kodak XAR-5 film for 72 h at −70°C. Blocking experiments using the Fn cell binding site tetrapeptide RGDS were carried out in a similar manner with the addition of 5 mg of RGDS (Calbiochem-Behring Corp., La Jolla, CA).

To determine whether gp120 was structurally related to the well characterized Fn receptors known as integrins, a Western blot was performed using a specific anti-β_1-integrin antiserum (kindly provided by Dr. Richard Hynes, Massachusetts Institute of Technology, Cambridge, MA) which had previously been shown to react with antigens from Candida, Drosophila, and human cell preparations (23). Nitrocellulose membranes containing *P. carinii* antigens were blocked as above, and then incubated with a 1:500 dilution of the primary antibody in TBS for 1 h. An ^{125}I-labeled anti–rabbit IgG secondary antibody (Amersham Corp., Arlington Heights, IL) was then used to label the primary antibody. The nitrocellulose membrane was then exposed to XAR-5 film as above to produce an autoradiogram.

Statistics. All results are expressed as mean±SEM. Statistical comparisons were performed by analysis of variance and Fisher's least significant difference for multiple pairwise comparisons. Statistical significance was accepted for $P < 0.05$.

Results

Initial studies resulted in the isolation and purification of *P. carinii* gp120 and production of a specific polyclonal anti-gp120 antibody. The purity of the isolated gp120 was assessed by both one-dimensional SDS-PAGE (Fig. 1) and two-dimensional electrophoresis (Fig. 2). The electroeluted sample of gp120 resolved into both a major band at 120 kD and a very small band at 50–55 kD. Both bands reacted with the anti-gp120 antibody by Western blot in the eluted gp120 solution as well as in the *P. carinii* organism lysate (Fig. 1). The gp120 purified by HPLC did not appear to contain the 55–60 kD component on either isoelectric focusing (Fig. 2) or on Western

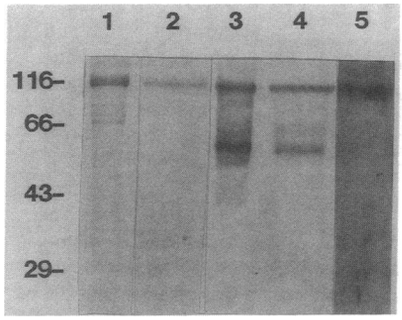

Figure 1. P. carinii antigens separated by SDS-PAGE and blotted with polyclonal anti-gp120. *P. carinii* were isolated by bronchoalveolar lavage and purified *P. carinii* lysates were separated by SDS-PAGE (lane *1*). *P. carinii* gp120 was subsequently purified by either electroelution from the original gel or by HPLC. SDS-PAGE of the eluted gp120 (lane *2*) demonstrated both gp120 and another small band at 55–60 kD. A Western blot of solubilized whole *P. carinii* antigens (lane *3*), the eluted gp120 (lane *4*), and HPLC purified gp120 (lane *5*) using polyclonal anti-gp120 demonstrated specific binding to both the gp120 and the 55–60-kD antigen in the whole *P. carinii* and eluted gp120 preparations, while the anti-gp120 bound only to the 120-kD band present in the HPLC purified gp120.

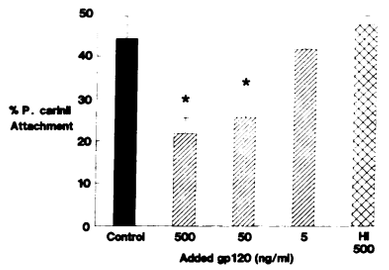

Figure 3. The effect of *P. carinii* gp120 on *P. carinii* attachment to A549 cells. Attachment of ^{51}Cr-labeled *P. carinii* to confluent monolayers of the alveolar epithelial cell line, A549, was quantified after a 4-h incubation in the presence or absence of soluble *P. carinii* gp120 or with the addition of heat-inactivated (*HI*) gp120 (100°C for 5 min). Results are expressed as mean±SEM. **P* < 0.01.

blot with the anti-gp120 antibody (Fig. 1). This suggests that the 50–55-kD band in the *P. carinii* lysate and in the eluted gp120 solution either shares similarly reactive epitopes or, more likely, is actually derived from degradation of the intact gp120 molecule.

Addition of eluted gp120 to the ^{51}Cr-labeled *P. carinii* attachment assay resulted in a significant, concentration-dependent decrease in *P. carinii* attachment (Fig. 3). This effect was maximum at 500 ng/ml (4 nM) which reduced attachment from 44.2±5.5% to 22.4±4.2% ($P < 0.01$), but persisted to concentrations as low as 20 ng/ml (0.16 nM). Release of ^{51}Cr by *P. carinii,* a sensitive marker of cellular injury (24), was not increased after the exposure of *P. carinii* to gp120. The HPLC-purified gp120 was an equipotent inhibitor of *P. carinii* attachment to A549 cells, indicating that the small quantity of 50–55-kD contaminant in the eluted gp120 solution did not contribute to its effects on *P. carinii* attachment. There was no evidence of disruption of the A549 cell monolayer during the 4 h of the attachment assay. Heat inactivation of gp120 (100°C × 5 min) eliminated the effect of gp120 on *P. carinii* attachment (Fig. 3). Control proteins eluted from polyacrylamide gels and purified in the same manner did not alter *P. carinii* attachment (data not shown).

The polyclonal anti-gp120 antibody was similarly examined for its effect on *P. carinii* attachment. As demonstrated for soluble gp120, anti-gp120 caused a significant, concentration-dependent decrease in *P. carinii* attachment (Fig. 4). Control rabbit sera or polyclonal rabbit IgG solutions did not affect *P. carinii* attachment to A549 cells. These data suggest that one function of gp120 may be to promote attachment of *P. carinii* to alveolar epithelial cells.

To evaluate the mechanism for the decrease in *P. carinii* attachment observed, the effects of soluble gp120 and anti-gp120 on ^{125}I-Fn binding to *P. carinii* organisms were assayed. Previous work from our laboratory has demonstrated that Fn binding to *P. carinii* organisms is a saturable and specific process that is essential for optimal *P. carinii* attachment (13). Addition of eluted gp120 produced a concentration-dependent decrease in ^{125}I-Fn binding to *P. carinii* (Fig. 5). The maximum inhibition of ^{125}I-Fn binding was caused by a gp120 concentration of 500 ng/ml, a concentration which also caused the greatest reduction in *P. carinii* attachment to A549 cells. The HPLC-purified gp120 solution had a similar effect on ^{125}I-Fn binding to *P. carinii.* Control eluted proteins had no effect on ^{125}I-Fn binding to *P. carinii* organisms.

The anti-gp120 antibody also produced a marked decrease in specific ^{125}I-Fn binding to *P. carinii* (Fig. 6) reaching its maximal effect at a 1:64 dilution. Control rabbit sera did not significantly alter ^{125}I-Fn binding to *P. carinii.* Therefore, it appears that the inhibition of *P. carinii* attachment to alveolar epithelial cells by gp120 and anti-gp120 may be due to their effects on Fn binding to the *P. carinii* organism.

To further examine the interaction between gp120 and Fn, *P. carinii* antigens separated by SDS-PAGE were blotted with ^{125}I-Fn. The autoradiogram demonstrated binding of ^{125}I-Fn to gp120 alone (Fig. 7), which provides direct evidence that gp120 acts as a specific binding site for Fn on the *P. carinii* organism. Previous characterization of Fn binding to *P. carinii* organisms had illustrated the binding was mediated by the RGD cell bind-

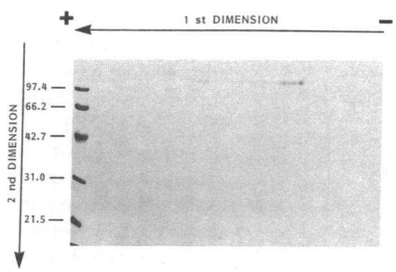

Figure 2. Two-dimensional gel electrophoresis of HPLC purified *P. carinii* gp120. *P. carinii* gp120 purified by HPLC on TSK 3000 SW sizing column was separated by 2-D gel with the first gel employing a pH range of 4–8 and the second gel containing 10% polyacrylamide. The figure demonstrates the multiple isoforms of gp120 previously described by Tanabe (10). There is no evidence of the 55–60-kD band present in the electroeluted gp120.

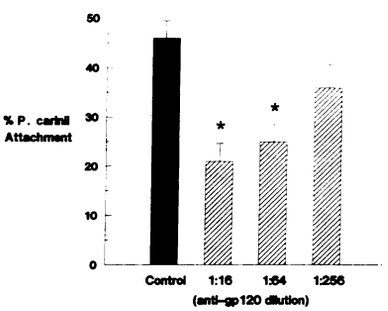

Figure 4. The effect of polyclonal anti-gp120 antibody on *P. carinii* attachment to A549 cells. Attachment of ^{51}Cr-labeled *P. carinii* to confluent monolayers of A549 cells was measured in the presence or absence of the indicated dilution of a polyclonal anti-gp120 antibody. Results are expressed as mean±SEM. **P* < 0.01.

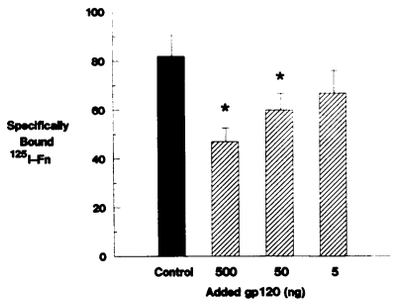

Figure 5. The effect of *P. carinii* gp120 on specific binding of ^{125}I-Fn to *P. carinii*. Specific binding of ^{125}I-Fn to *P. carinii* was quantified after a 1-h incubation of *P. carinii* organisms with 3 μg of ^{125}I-Fn in the presence or absence of 125 μg of unlabeled Fn. Binding of ^{125}I-Fn to *P. carinii* was subsequently measured after the addition of soluble *P. carinii* gp120. Results are expressed as mean±SEM. $^*P < 0.01$.

ing site on the Fn molecule (13). Addition of a synthetic RGDS tetrapeptide effectively decreased the binding of ^{125}I-Fn to gp120 (Fig. 8). This suggests the Fn binding site on *P. carinii* may be similar to the Fn binding site present on integrins (25, 26). To determine whether gp120 displayed structural homology with known integrins, a Western blot of *P. carinii* antigens with a specific anti-β_1-integrin antiserum was performed. The Western blot exhibited binding of the anti-β_1-integrin antiserum to gp120 alone among the *P. carinii* antigens (Fig. 7). Thus, gp120 is an RGD-dependent Fn binding protein that appears to bear structural similarities to one subunit of the integrins.

Discussion

The attachment of *P. carinii* to alveolar epithelial cells is thought to be required for the development of subsequent infection. These data provide the first information regarding the role of *P. carinii* gp120 in attachment of *P. carinii* organisms to alveolar epithelial cells. Both soluble gp120 and anti-gp120 antibodies caused a significant decrease in *P. carinii* attachment to A549 cells, and also markedly reduced specific binding of Fn to the organism. Furthermore, gp120 was the only apparent site of Fn binding in solubilized *P. carinii* preparations.

Attachment of pathogenic organisms to host cells is a critical initial step in the development of many infections (27, 28). Many pathogens exhibit specific attachment antigens or factors on their cell surface, which may act either by attaching directly to host cells (29) or by binding to host-derived cell-adhesive proteins and using these proteins as a bridge for attachment to host cells (30). Fibronectin, an extracellular matrix protein known to mediate cell attachment (31, 32), has been demonstrated to aid in the adherence of many organisms, including

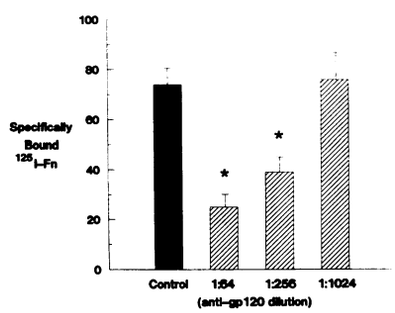

Figure 6. The effect of polyclonal anti-gp120 on specific binding of ^{125}I-Fn to *P. carinii*. Specific binding of ^{125}I-Fn was quantified as in Fig. 4, and binding measurements were repeated in the presence of polyclonal anti-gp120 antibody. Results are expressed as mean±SEM. $^*P < 0.001$.

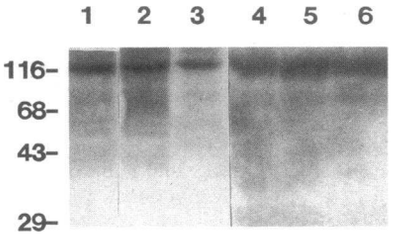

Figure 7. Autoradiograms of ^{125}I-Fn and anti-β_1-integrin antibody binding to *P. carinii* gp120. *P. carinii* lysates (lanes *1–3*) were separated by SDS-PAGE and transferred to nitrocellulose. The nitrocellulose membranes were blotted with 2.5 μCi of ^{125}I-Fn for 4 h and exposed to XAR-5 film at −70°C. The autoradiogram demonstrates the site of ^{125}I-Fn binding corresponding to gp120. *P. carinii* lysates (lanes *4–6*) were separated as above, and blotted with a polyclonal anti-β_1-integrin antibody which was imaged using a ^{125}I-labeled secondary antibody. The autoradiogram demonstrates binding of the anti-β_1-integrin antibody to *P. carinii* gp120.

Staphylococcus aureus (33), streptococci (34), *Treponema pallidum* (35), *Candida albicans* (36), and *Trypanasoma* sp (37). Previous work from our laboratory has established that Fn binds to *P. carinii* in a saturable and specific manner and is required for optimal attachment of the organism (13).

The interaction between *P. carinii* and alveolar cells is poorly understood. In vitro studies with *P. carinii* have been hampered by the inability to grow the organism in continuous culture. The major cellular constituents of *P. carinii* have only recently been determined. The major antigen of *P. carinii*, gp120, is a heavily glycosylated membrane glycoprotein. The surface location and relative abundance of this molecule make it likely that gp120 is an integral component in the interaction of the organism with host cells. Our data suggest that an important function of gp120 is to promote *P. carinii* attachment to the alveolar epithelium by acting as a Fn binding site on the organism. Fn binding proteins have been isolated from other pathogenic organisms, and the expression of these proteins facilitates attachment of the organisms to host cells (30). *P. carinii* appear to be similarly dependent upon gp120 to enhance

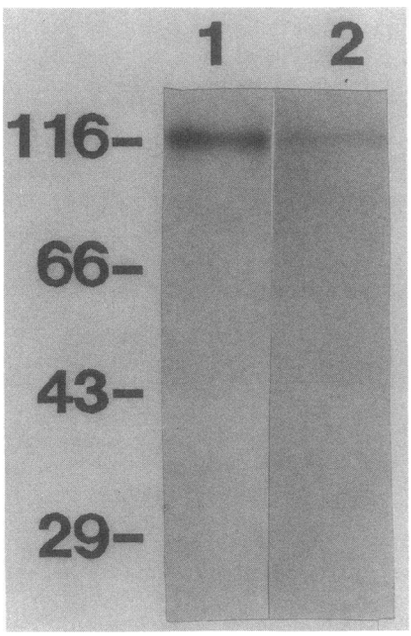

Figure 8. Autoradiogram demonstrating inhibition of ^{125}I-Fn binding to *P. carinii* gp120 by addition of the Fn cell binding site analogue RGDS. Purified *P. carinii* gp120 was separated by SDS-PAGE and transferred to nitrocellulose. The nitrocellulose membranes were then blotted with 2.5 μCi of ^{125}I-Fn alone (lane *1*) or with ^{125}I-Fn plus 5 mg of RGDS (lane *2*), and the autoradiograms developed as previously described. Addition of RGDS resulted in a marked decrease in ^{125}I-Fn binding to gp120.

attachment of the organism to alveolar cells. However, it is clear from these studies that *P. carinii* organisms likely possess other mechanisms for attachment to host cells, as inhibition of gp120 mediated attachment did not completely abolish *P. carinii* attachment.

Integrins are membrane spanning proteins made up of distinct α and β subunits that function as cellular receptors for extracellular matrix molecules. Integrins, by providing a link between the cellular cytoskeleton and the extracellular matrix, are essential components in cell–cell and cell–matrix attachment. The β-subunits of the integrins are highly conserved with similarly structured molecules present on insect (38), avian (39), and human cells (39). A recent report of binding of an anti-β_1-integrin antibody to an uncharacterized 95-kD antigen on *Candida albicans* (23) extends the range of organisms on which integrin-like molecules may be present. Our data which demonstrate binding of anti-β_1-integrin to *P. carinii* gp120 provide further possible evidence for the evolutionary conservation of this molecule. Future studies to determine the amino acid sequence of gp120 will permit a direct comparison between the structures of gp120 and β_1-integrin.

The role of *P. carinii* antigens in the attachment of *P. carinii* to alveolar epithelial cells has not been previously investigated. Our data suggest that the *P. carinii* membrane glycoprotein gp120 functions as a Fn binding protein and mediates attachment of the organism to alveolar cells, an essential initial step in the development of *P. carinii* infection. Further investigation into the mechanism of *P. carinii* attachment may lead to the development of novel therapeutic strategies to block attachment of the organism and thereby reduce the incidence of this often fatal disease.

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