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# *Pneumocystis carinii* Attachment Increases Expression of Fibronectin-Binding Integrins on Cultured Lung Cells

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***Pneumocystis carinii* is an extracellular pathogen which requires attachment to alveolar epithelial cells for growth and replication. Previous studies have demonstrated that the extracellular matrix protein fibronectin (Fn) facilitates attachment of *P. carinii* to lung cells. This study addresses the role of cell surface Fn receptors (integrins) as mediators of *P. carinii* attachment and demonstrates the effect of *P. carinii* attachment on integrin expression on cultured lung cells. To determine the role of Fn-binding integrins in *P. carinii* attachment, attachment of $^{51}$Cr-labelled *P. carinii* organisms to the lung epithelial cell line A549 was quantified in the presence or absence of anti-integrin antibodies. Antibodies to the $\alpha_v$ and $\alpha_5$ integrin subunits significantly inhibited *P. carinii* attachment, while addition of antibody to the $\alpha$ subunit of a non-Fn-binding integrin, $\alpha_2$, did not affect *P. carinii* attachment. To further investigate the role of Fn-binding integrins in *P. carinii* attachment, the effect of *P. carinii* attachment on expression of the $\alpha_v$ and $\alpha_5$ integrin subunits was determined. A549 cells incubated with either *P. carinii* organisms or with the *P. carinii* major surface glycoprotein gp120 demonstrated a 3- to 10-fold increase in expression of the $\alpha_5$ integrin subunit; however, neither *P. carinii* nor gp120 affected the expression of $\alpha_v$ integrin. Furthermore, the effects of *P. carinii* on A549 cell $\alpha_5$ integrin expression were attenuated by the addition of an anti-gp120 antibody which blocks *P. carinii* attachment to A549 cells. Therefore, *P. carinii* attachment to lung epithelial cells appears to be mediated by $\alpha_v$- and $\alpha_5$-containing integrins expressed on the epithelial cell surface, and *P. carinii* attachment results in increased expression of the $\alpha_5$ integrin subunit.**

*Pneumocystis carinii* is a major cause of morbidity and mortality in immunocompromised hosts, especially those with AIDS (6, 19). *P. carinii* is an exclusively extracellular pathogen which adheres tightly to the alveolar epithelium, with a preference for binding to type I alveolar epithelial cells (16, 35, 36). Current concepts suggest that attachment to host cells is a requirement for growth and replication of the organism (7, 9, 29). Electron microscopy has shown tight interdigitation of the cell membranes of *P. carinii* and alveolar epithelial cells, but there is no evidence of membrane fusion or intracellular invasion (12, 18, 35, 36).

Previous studies have demonstrated a role for the extracellular matrix protein fibronectin (Fn) as a mediator of *P. carinii* attachment to host cells (20–23). Fn binds specifically to the *P. carinii* major surface glycoprotein gp120 (22), a 110- to 120-kDa highly glycosylated surface glycoprotein (11, 15, 26). Surface-bound Fn can then act as a bridge between the *P. carinii* organism and host cell surface Fn receptors.

Several cell surface Fn receptors belonging to the integrin family of cell membrane-spanning proteins have been identified (28). Integrins are heterodimeric molecules consisting of separate $\alpha$ and $\beta$ chains which act as mediators of cell-matrix and cell-cell attachment (1, 30). The integrins $\alpha_5\beta_1$, $\alpha_v\beta_1$, and $\alpha_v\beta_6$ have all been described previously to act as Fn receptors on epithelial cells (4, 25, 31); however, no previous studies have identified the Fn-binding integrins present on alveolar epithelial cells. Histochemical studies have demonstrated that cell-associated Fn is present on the surface of type I cells in vivo but not on the surface of type II cells (27). Fn binds to both the luminal and basal surfaces of the type I cell (27), suggesting that it may function both as a mediator of type I cell interaction with intra-alveolar cells and in type I cell attachment to the basal lamina.

The current study addresses the role of lung epithelial cell surface Fn-binding integrins in the attachment of *P. carinii* to host cells. In vitro attachment of *P. carinii* to the lung epithelial cell line A549 was inhibited by antibodies to the $\alpha_v$ and $\alpha_5$ integrin subunits, which represent $\alpha$ subunits of Fn-binding integrins. In addition, incubation of A549 cells with either *P. carinii* or gp120 resulted in a significant increase in expression of the $\alpha_5$ integrin subunit, an effect which was inhibited by addition of attachment-blocking anti-gp120 antibodies. In contrast to the increase in $\alpha_5$ expression, A549 cell surface $\alpha_v$ subunit expression was not affected by either *P. carinii* or gp120.

## MATERIALS AND METHODS

**Isolation of *P. carinii*.** *P. carinii* pneumonia was induced in pathogen-free rats by immunosuppression with dexamethasone and transtracheal inoculation of *P. carinii* trophozoites as described previously (20–23). Rats received water containing dexamethasone (2 mg/ml), tetracycline (500 μg/ml), and nystatin (200,000 U/ml) ad libitum. Rats were inoculated transtracheally with $10^6$ *P. carinii* trophozoites after 5 to 7 days of immunosuppression. *P. carinii* trophozoites were harvested 6 to 8 weeks following inoculation, when the rats had severe *P. carinii* pneumonia. The rats were sacrificed, and the tracheas were cannulated. The lungs were lavaged six times with 8-ml aliquots of Hanks balanced salt solution plus 0.6 mM EDTA, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (4 μg/ml), and amphotericin B (0.5 μg/ml). Approximately 40 to 45 ml of lavage fluid was obtained from each rat. The lavage

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fluid was centrifuged (200 × *g*, 10 min) to pellet inflammatory and alveolar cells, and the supernatant was saved. The pellet was resuspended to prepare cytopreparation smears on a cytologic centrifuge (Cytospin II; Shandon Southern Instruments). The presence of *P. carinii* cysts and trophozoites was verified by use of Gomori methenamine silver stain (3) and Diff-Quik stain (5). To obtain a highly purified population of *P. carinii* trophozoites, the supernatant was centrifuged (1,400 × *g*, 30 min) and resuspended in 1 ml of lavage fluid, and *P. carinii* trophozoites were quantified by the method of Bartlett et al. (2). A typical rat yielded $10 \times 10^6$ to $20 \times 10^6$ trophozoites. Examination of the trophozoite suspension showed that *P. carinii* organisms represented 97 to 98% of the cellular material in the suspension. Any samples containing bacterial, fungal, or inflammatory cell contamination were discarded.

***P. carinii* adherence assay.** *P. carinii* adherence to an alveolar epithelial cell line was quantified by use of our previously described method (20–22). Freshly isolated *P. carinii* trophozoites were incubated for 18 h in 2 ml of Dulbecco modified Eagle medium (DMEM) containing 0.5 ml of fetal calf serum (FCS) and 50 μCi of $^{51}$Cr-sodium chromate (New England Nuclear, Boston, Mass.). Following incubation, the $^{51}$Cr-labelled *P. carinii* suspension was centrifuged (1,400 × *g*, 15 min), the supernatant was discarded, and the pellet was resuspended in DMEM plus 10% FCS. The *P. carinii* suspension was washed four times to remove unincorporated $^{51}$Cr and resuspended in DMEM at $20 \times 10^6$ *P. carinii* organisms per ml.

The lung epithelial cell line A549 (ATCC 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 DMEM plus 10% FCS on 24-well tissue culture dishes. To determine the effect of specific polyclonal antibodies to the $\alpha_2$, $\alpha_5$, and $\alpha_v$ integrin subunits (Chemicon, La Jolla, Calif.) on *P. carinii* adherence, A549 cell monolayers were preincubated for 1 h in either DMEM alone or DMEM plus 10 μg of the anti-integrin antibodies per ml. $^{51}$Cr-labelled trophozoites ($2 \times 10^6$) were then added to each well of A549 cells, and the wells were incubated at 37°C for 4 h. Following incubation, the medium, containing nonadherent trophozoites, was removed and saved. The cell monolayer, containing bound trophozoites, was disrupted with 10% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) and saved. $^{51}$Cr-labelled *P. carinii* organisms were quantified in each fraction (Beckman model 5500 gamma counter), and percent attachment was expressed as follows: percent attachment = $[A/(A + B)] \times 100$, where $A$ is $^{51}$Cr-labelled *P. carinii* bound to the A549 monolayer and $B$ is $^{51}$Cr-labelled *P. carinii* free in the medium. All experiments were performed in triplicate and repeated on four separate *P. carinii* isolates.

**Isolation and purification of *P. carinii* gp120.** The purification of *P. carinii* gp120 is based on the Fn-binding characteristics of gp120. *P. carinii* organisms were purified from bronchoalveolar lavage specimens of rats with fulminant *P. carinii* pneumonia. The organisms were then incubated with Zymolyase (1 mg/ml) to free the gp120 from the cell membrane. The remaining cellular material was then pelleted, and the gp120-rich supernatant was applied to an Fn-Sepharose column prepared by standard methods. The column was then washed and the gp120 was eluted with a 50 mM EDTA elution buffer. This procedure yielded 2 to 5 μg of gp120 per *P. carinii*-infected rat. The purity of the gp120 was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein staining with Coomassie blue and subsequently by Western blotting (immunoblotting) with polyclonal antibodies to gp120 developed in our laboratory.

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

**Isolation of A549 cell membrane integrins.** A549 cells were grown to confluency in 100-mm-diameter culture plates in DMEM plus 10% FCS. The medium was then changed to DMEM alone, and 24 h later, *P. carinii* organisms ($40 \times 10^6$) or gp120 (1 or 10 μg/ml) was added. Following an additional 24-h incubation period, A549 cell membrane fractions were obtained. The cells were washed three times with ice-cold phosphate-buffered saline (PBS) plus 20 mM EDTA and then incubated for 30 min in PBS plus EDTA to detach the cells from the plates. Following detachment, the cells were pelleted by centrifugation (200 × *g*, 5 min) and resuspended in cell lysis buffer (100 mM Tris, 100 mM octylglucoside [pH 7.5]). Following a 5-min incubation on ice, cell fragments were pelleted by centrifugation (12,000 × *g*, 5 min), and the solubilized cell membrane integrins in the supernatant were saved for subsequent Western blotting.

Additional control experiments were performed in a manner similar to that described above following addition of *Escherichia coli* lipopolysaccharide (LPS; 1 to 100 μg/ml; Sigma) or the anti-gp120 antibody described above at a 1:64 dilution.

**Western blots of A549 cell membrane preparations.** Ten-microgram aliquots of the A549 cell membrane preparations were separated by nonreducing SDS-PAGE on 8% polyacrylamide gels. Proteins were then transferred to nitrocellulose membranes and blotted with anti-$\alpha_5$ or anti-$\alpha_v$ antibodies (Chemicon). Secondary antibodies conjugated to alkaline phosphatase were then added and visualized with a chemiluminescent substrate reaction (Bio-Rad Laboratories). The relative intensities of bands visualized on the Western blots were quantified by densitometry (Bio-Rad model GS-670 densitometer).

**Northern (RNA) blotting of A549 RNA.** A549 cells were grown to confluency in 100-mm-diameter tissue culture plates and then incubated in the presence or absence of *P. carinii* as described previously. Total cellular RNA was isolated from A549 cells by standard methods. Twenty-microgram samples of A549 cell RNA were separated by electrophoresis on an agarose-formaldehyde gel and transferred to a nitrocellulose membrane. The membranes were then blotted with a $^{32}$P-labelled $\alpha_5$ integrin cDNA probe (Gibco BRL). Expression of the constitutively expressed housekeeping gene CHO-B was used to control for equal loading of RNA. Relative levels of $\alpha_5$ integrin mRNA were quantified by densitometry as described previously.

**Statistics.** Results are expressed as means ± standard errors of the means. Statistical analysis of the data was completed by one-way analysis of variance, and pairwise comparisons were performed by Fisher's least significant difference test. Statistical significance was accepted for a $P$ of $<0.01$.

## RESULTS

Initial experiments were undertaken to determine the role of Fn-binding integrins expressed in A549 cells in *P. carinii* attachment. Previous studies have demonstrated that A549 cells express both $\alpha_v$ and $\alpha_5$ integrins (17), which constitute the α subunits of the $\alpha_v\beta_1$, $\alpha_v\beta_6$, and $\alpha_5\beta_1$ Fn-binding integrins.

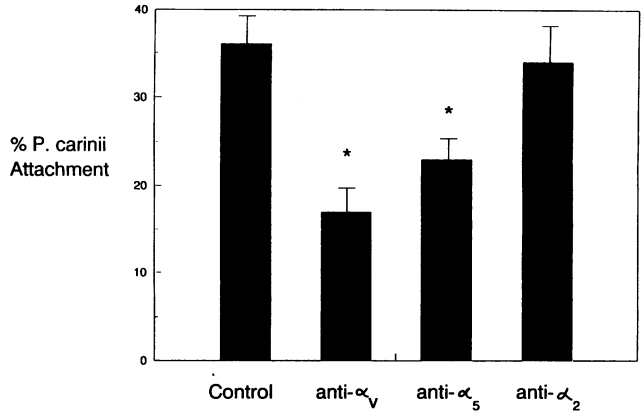


FIG. 1. Inhibition of *P. carinii* attachment to A549 cells by anti-$\alpha_v$ and anti-$\alpha_5$ antibodies. Attachment of $^{51}$Cr-labelled *P. carinii* to the lung epithelial cell line A549 was significantly decreased by addition of antibodies to the Fn-binding integrins $\alpha_v$ and $\alpha_5$. In contrast, addition of anti-$\alpha_2$ integrin antibodies did not affect *P. carinii* attachment to A549 cells. *, $P < 0.01$.

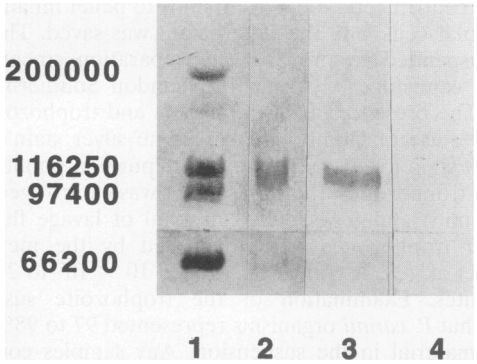


FIG. 2. SDS-polyacrylamide gel and Western blot demonstrating the purity of isolated *P. carinii* gp120. *P. carinii* gp120 was isolated as described in the text and subjected to SDS-PAGE on a 10% polyacrylamide gel. Purified gp120 migrates as a single band of 110 to 120 kDa (lane 2). A Western blot of purified gp120 with a polyclonal antibody developed against gp120 also demonstrates a single specific band (lane 3). Preimmune serum showed no reactivity with gp120 (lane 4). Molecular weight markers are illustrated in lane 1.

Therefore, specific polyclonal antibodies to the $\alpha_v$ and $\alpha_5$ integrins were utilized to block *P. carinii* attachment to A549 cells (Fig. 1). Preincubation of A549 cells with the anti-$\alpha_v$ antibody decreased *P. carinii* attachment from 36.3% ± 2.7% to 16.9% ± 2.1% ($P < 0.01$). Similarly, preincubation of the A549 cells with the anti-$\alpha_5$ antibody decreased attachment to 23.4% ± 2.9% ($P < 0.01$ compared with control). Control experiments performed with polyclonal antibodies to the non-Fn-binding integrin subunit $\alpha_2$ resulted in no significant effect on *P. carinii* attachment (34.4% ± 3.7%; $P > 0.2$ compared with control). These data provide the first evidence for the role of lung cell surface integrin receptors as mediators of *P. carinii* attachment.

Further studies were then performed to determine whether *P. carinii* organisms or *P. carinii* gp120 affected the expression of the $\alpha_v$ and $\alpha_5$ integrin subunits on A549 cells. gp120 was purified from *P. carinii* organisms (22) and used to develop polyclonal rabbit anti-*P. carinii* antibodies. The purity of the gp120 and specificity of the antibodies were assessed by SDS-PAGE and Western blotting, respectively (Fig. 2).

To assess the effect of *P. carinii* organisms on integrin expression in A549 cells, the A549 cells were incubated in the presence or absence of *P. carinii* organisms for 24 h. Following this incubation period, the cells were washed and the cell membrane fractions were purified by standard methods, and equal quantities of protein were run on SDS-polyacrylamide gels. Western blotting of these gels with anti-$\alpha_v$ and anti-$\alpha_5$ antibodies was performed to determine whether *P. carinii* binding to the A549 cells caused a significant increase in expression of these integrin subunits.

The anti-$\alpha_5$ Western blot (Fig. 3) demonstrated that *P. carinii*-treated A549 cells exhibited a threefold increase in the expression of the $\alpha_5$ integrin subunit compared with untreated cells. In contrast, *P. carinii* treatment resulted in no significant change in expression of the $\alpha_v$ subunit (Fig. 4). Control experiments using equal numbers of washed rat erythrocytes demonstrated no effect on A549 integrin expression. Therefore, the observed effects on integrin expression appear to be due to *P. carinii* and are relatively specific for the $\alpha_5$ integrin subunit.

Previous work in our laboratory has demonstrated that the major *P. carinii* surface glycoprotein gp120 functions as an Fn-binding site on the *P. carinii* cell surface and is an important mediator of *P. carinii* attachment (22). To determine whether the effects of intact *P. carinii* organisms on $\alpha_5$ and $\alpha_v$ expression in A549 cells were mediated by gp120, purified gp120 was added to A549 cells at low (1 μg/ml) or high (10 μg/ml) concentrations for 24 h. Addition of gp120 resulted in a 6-fold (1 μg/ml) or 10-fold (10 μg/ml) increase in $\alpha_5$ integrin subunit expression (Fig. 3), corresponding to the increased $\alpha_5$ expression caused by intact *P. carinii* organisms. In addition, gp120 failed to affect the expression of the $\alpha_v$ integrin subunit in A549 cells (Fig. 4). These data indicate that the *P. carinii* surface glycoprotein gp120 affects cell surface integrin expression in a manner similar to that of intact *P. carinii* organisms.

Although no bacterial organisms were detected in any of the *P. carinii* preparations used in these studies, there was concern

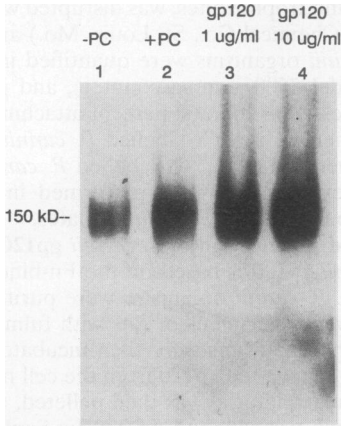


FIG. 3. Effect of *P. carinii* or gp120 on expression of $\alpha_5$ integrin subunit in A549 cells. A549 cells were incubated for 24 h in the absence (−PC) or presence (+PC) of *P. carinii* organisms or with 1 or 10 μg of the *P. carinii* surface glycoprotein gp120 per ml. A549 cell membrane extracts were then separated by SDS-PAGE and blotted with anti-$\alpha_5$ integrin antibodies. Addition of *P. carinii* (lane 2) resulted in a threefold increase in $\alpha_5$ expression, while addition of gp120 resulted in a 6-fold (lane 3) or 10-fold (lane 4) increase in $\alpha_5$ expression compared with control A549 cells (lane 1).

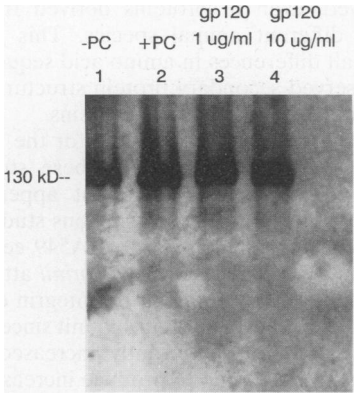


FIG. 4. Effect of *P. carinii* or gp120 on expression of $\alpha_v$ integrin subunit in A549 cells. A549 cells were treated as described in the legend to Fig. 2, except that the cell membrane extracts were blotted with an anti-$\alpha_v$ integrin antibody. Addition of *P. carinii* (lane 2) or gp120 (lanes 3 and 4) resulted in no significant change in the expression of the $\alpha_v$ integrin subunit compared with control A549 cells (lane 1).

about the possibility of contamination of the samples with bacterial LPS. To address this, the effects of LPS on expression of the $\alpha_v$ and $\alpha_5$ integrin subunits in A549 cells were determined. LPS (100 μg/ml) did not affect expression of either the $\alpha_v$ (Fig. 5a) or $\alpha_5$ (Fig. 5b) integrin subunits following 24-h incubations with A549 cells. Therefore, it is unlikely that LPS contamination of the *P. carinii* preparations contributed to the *P. carinii*-induced changes in integrin expression noted previously.

Further experiments were performed to determine whether *P. carinii* attachment to A549 cells was necessary for the organism to affect expression of the $\alpha_5$ integrin subunit. A549 cells were incubated with *P. carinii* organisms in the presence or absence of anti-gp120 antibodies, which have been determined previously to inhibit *P. carinii* attachment to A549 cells (22). Consistent with prior experiments, addition of *P. carinii* organisms resulted in a fourfold increase in $\alpha_5$ expression by Western blot analysis (Fig. 6). Addition of the attachment-

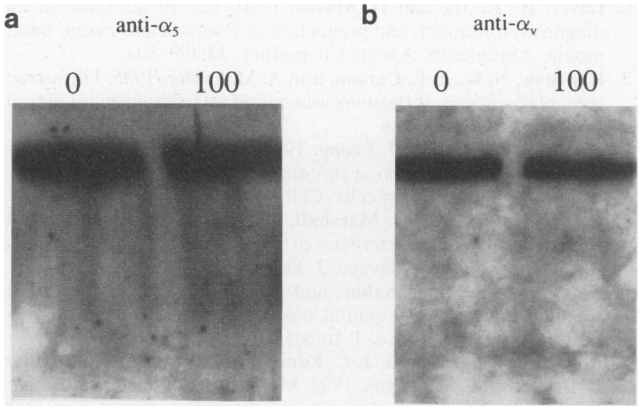


FIG. 5. Effect of bacterial LPS on expression of $\alpha_5$ and $\alpha_v$ integrin subunits in A549 cells. A549 cells were incubated in the absence (0) or presence (100) of 100 μg of LPS per ml. Western blots of A549 cell membrane extracts with anti-$\alpha_5$ (a) or anti-$\alpha_v$ (b) antibodies demonstrated no change in the expression of the $\alpha_5$ or $\alpha_v$ integrin subunits in A549 cells exposed to LPS compared with untreated A549 cells.

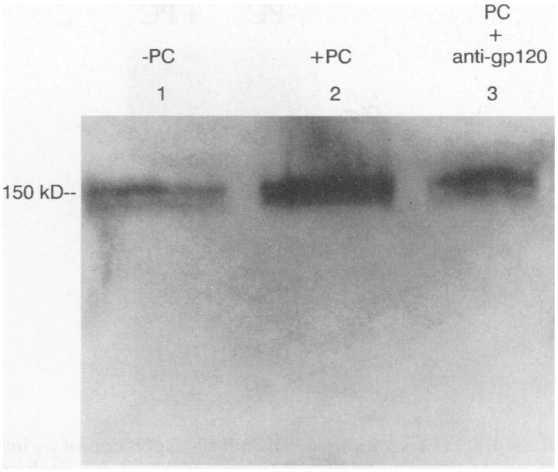


FIG. 6. Attenuation of *P. carinii*-induced increase in $\alpha_5$ integrin expression in A549 cells by blocking *P. carinii* attachment with anti-gp120 antibodies. A549 cells were cultured in the absence (−PC) or presence (+PC) of *P. carinii* organisms or in the presence of *P. carinii* plus anti-gp120. Western blotting of A549 cell membrane extracts with anti-$\alpha_5$ antibodies demonstrates a fourfold increase in $\alpha_5$ integrin subunit expression in A549 cells exposed to *P. carinii* (lane 2) compared with unexposed cells (lane 1). However, addition of *P. carinii* with anti-gp120 (lane 3) resulted in a 50% decrease in $\alpha_5$ expression compared with cells exposed to *P. carinii* alone (lane 2).

blocking antibodies to gp120 attenuated this effect, resulting in a 50% decrease in the *P. carinii*-stimulated expression (Fig. 6). Preimmune rabbit serum did not affect the *P. carinii*-induced increase in $\alpha_5$ integrin expression. Further control experiments with the anti-gp120 antibody alone demonstrated no effect of the antibody by itself on $\alpha_5$ expression in A549 cells (data not shown). Although the anti-gp120 did not completely block the *P. carinii*-mediated increase in $\alpha_5$ expression, these data provide further evidence for the role of gp120 and *P. carinii* attachment to A549 cells in mediating the effect of *P. carinii* organisms on $\alpha_5$ integrin expression.

To determine a possible mechanism for the *P. carinii*-induced increase in $\alpha_5$ integrin expression on A549 cells, the effect of *P. carinii* on $\alpha_5$ mRNA expression was investigated. Northern blots of RNA isolated from A549 cells in the absence or the presence of *P. carinii* organisms demonstrated no change in the expression of $\alpha_5$ integrin mRNA (Fig. 7). Therefore, it is unlikely that the elevated levels of $\alpha_5$ cell surface expression are due to an increased rate of transcription of the $\alpha_5$ integrin gene.

## DISCUSSION

This study addresses the role of lung cell surface integrins in the pathogenesis of *P. carinii* infection. The data indicate that inhibition of either $\alpha_v$- or $\alpha_5$-containing integrins results in a significant decrease in *P. carinii* attachment to the lung epithelial cell line A549. *P. carinii* organisms are also able to stimulate the expression of the $\alpha_5$ integrin subunit but do not appear to affect the expression of the $\alpha_v$ subunit. In addition, this effect can be duplicated by addition of the *P. carinii* surface glycoprotein gp120. Furthermore, inhibition of *P. carinii* attachment to A549 cells significantly decreases the effect of *P. carinii* on $\alpha_5$ integrin expression.

The potential role of cell surface integrins in the pathogenesis of microbial infection has not been investigated thor-

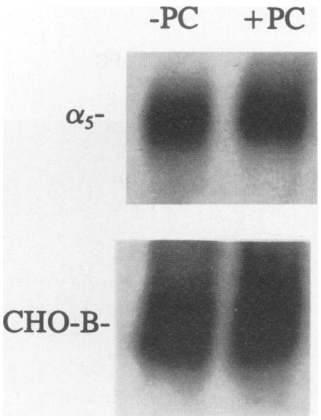


FIG. 7. Effect of addition of *P. carinii* on expression of $\alpha_5$ integrin mRNA in A549 cells. A549 cells were incubated for 24 h in the presence or absence of *P. carinii* organisms, and total cellular RNA was isolated from the A549 cells. Following agarose-formaldehyde electrophoresis, the RNA was transferred to a Nytran membrane and blotted with $^{32}$P-labelled $\alpha_5$ integrin cDNA. A cDNA probe of the constitutively expressed gene CHO-B was used to control for the equivalence of RNA loading.

oughly. Previous studies have shown that the intracellular pathogen *Yersinia pseudotuberculosis* possesses a cell surface molecule known as invasin, which binds directly to several $\beta_1$-containing integrins, including the $\alpha_5\beta_1$ integrin, and facilitates binding and uptake of the organism (8). Although several studies have examined the role of extracellular matrix constituents such as Fn or vitronectin as attachment factors for pathogenic organisms (10, 14, 24, 34), these studies have not examined the role of host cell surface integrins in pathogen attachment.

The data presented extend our previous observations regarding the role of Fn as a mediator of *P. carinii* attachment. If, as postulated by our previous work, Fn acts as a bridge between *P. carinii* organisms and the alveolar epithelium, then it follows that alveolar cell surface Fn receptors are also an essential component of *P. carinii* attachment. The best-studied Fn receptors are the integrins, a family of heterodimeric cell membrane-spanning molecules which form a bridge between the extracellular matrix and the cell cytoskeletal network (1, 21, 28). Although Fn binds to several members of the integrin family, these studies have focused on Fn-binding integrins containing the $\alpha_v$ and $\alpha_5$ integrin subunits since these Fn-binding integrins are commonly present on epithelial cells.

These data demonstrate that *P. carinii* organisms increase expression of the $\alpha_5$ integrin subunit, which represents a potential cell surface binding site for the organism. The ability of a pathogen to increase expression of its host cell surface binding site has not been reported previously. Our data indicate that *P. carinii* attachment, likely through its surface glycoprotein gp120, is required for the observed *P. carinii*-induced increase in $\alpha_5$ integrin expression. Therefore, it is unlikely that soluble factors secreted by *P. carinii* organisms or present in the *P. carinii* preparations are responsible for the observed effects.

*P. carinii* gp120 glycoproteins from several animal sources (11, 32, 33) have recently been cloned and determined to actually represent a family of as many as 100 separate homologous proteins. The gp120 family of proteins are approximately 70% homologous at the amino acid level. The proteins are high in cysteine content, and the cysteine residues are highly conserved, even in proteins derived from *P. carinii* isolated from different animal species. This suggests that, despite the small differences in amino acid sequences, there is a strongly conserved secondary protein structure that may be responsible for the function of the proteins.

The cellular mechanisms responsible for the increase in $\alpha_5$ integrin expression demonstrated in these studies are unknown; however, *P. carinii* attachment appears to be an essential initiator of the response. Previous studies examining *P. carinii*-induced growth inhibition of A549 cells have demonstrated a similar requirement for *P. carinii* attachment (13). It appears that the observed effects on integrin expression are relatively specific for the $\alpha_5$ integrin subunit since expression of the $\alpha_v$ subunit was not significantly increased. Because *P. carinii* attachment is required to produce increased $\alpha_5$ integrin expression, it is possible that *P. carinii* attachment to cell surface integrins results in the necessary intracellular signal needed for increased $\alpha_5$ expression to occur. Alternatively, the increase in $\alpha_5$ expression may be related to a more general cellular response to *P. carinii* infection, which requires only attachment as an initial step.

The identification of cell surface integrins which mediate *P. carinii* attachment to lung cells provides more insight into the pathogenesis of *P. carinii* pneumonia. By binding to cell surface receptors and increasing their cell surface expression, *P. carinii* organisms may be able to modulate their ability to attach to host cells, and this may help to explain the rapid proliferation of *P. carinii* organisms in vivo. Further investigation of the interactions between lung epithelial cells and *P. carinii* organisms will be required to define the specific cellular signals involved in this process.

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