

Purification and Characterization of a Major Human *Pneumocystis carinii* Surface Antigen

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Abstract

Previous studies of *Pneumocystis carinii* have identified the major surface antigen of rat and human isolates as proteins of 116,000 and 95,000 mol wt, respectively, that are antigenically not identical. In this study both rat and human *P. carinii* proteins were purified by solubilization with zymolyase followed by molecular sieve and ion exchange chromatography. The native proteins had an apparent mol wt of 290,000 or greater, based on molecular sieve studies as well as cross-linking studies. Both proteins were glycoproteins; treatment with endoglycosidase H resulted in a 9% decrease in mol wt. The carbohydrate composition of the rat *P. carinii* glycoprotein was distinct from the human isolate; glucose, mannose, galactose, and glucosamine occurred in approximately equimolar ratios in the human *P. carinii* protein, whereas glucose and mannose were the predominant sugars of the rat *P. carinii* protein. To evaluate humoral immune responses to the human *P. carinii* protein, an enzyme-linked immunosorbent assay using purified protein was developed. Some, but not all, patients who subsequently developed *P. carinii* pneumonia demonstrated a serum antibody response to the surface antigen. Nearly all subjects without a history of *P. carinii* pneumonia had no detectable antibodies. Purified *P. carinii* proteins will greatly facilitate the investigation of host-*P. carinii* interactions. (*J. Clin. Invest.* 1991; 87:163-170.) Key words: AIDS • immunosuppression • *Pneumocystis carinii* pneumonia • gp95 • ELISA

Introduction

Pneumocystis carinii pneumonia is a major, life-threatening complication of immunodeficiency diseases, especially AIDS (1). Although the incidence of this disease has increased dramatically in the past decade, *P. carinii*-host interactions remain poorly understood, and investigations into such interactions have primarily used organisms obtained from a rat model of *P. carinii* pneumonia. Antigens specific for *P. carinii* have recently been identified by the immunoblot technique, and antigenic differences between rat and human isolates have been demonstrated using monoclonal and polyclonal antibodies (2-6). Use of *P. carinii*-specific antigens in further studies has been limited by difficulties in purifying these antigens.

Studies of rat and ferret *P. carinii* have identified the major surface antigen as a glycoprotein with a molecular weight as

determined by SDS-PAGE of 110,000-120,000; this protein is frequently referred to as gp116¹ (7-11). There appears to be an analogous protein in human *P. carinii* with a mol wt of ~95,000-98,000 that is the major protein based on Coomassie-blue staining of SDS-PAGE gels (2, 4). Our laboratory has developed a monoclonal antibody (6B8) to this protein, and immunofluorescence studies have documented that this is a surface antigen (3). Moreover, this protein is different from the analogous rat *P. carinii* protein since 6B8 does not react with the rat *P. carinii* antigen (3). Since studies investigating the interaction of *P. carinii* with humans should use antigens derived from human *P. carinii*, we undertook to purify and characterize this human *P. carinii* surface antigen, and to develop an ELISA to measure antibody responses to this antigen. To obtain further information about the relationship between rat and human *P. carinii*, we simultaneously characterized the rat *P. carinii* antigen.

Methods

Organisms. Rat *P. carinii* were obtained from lungs of Sprague-Dawley rats treated with dexamethasone for 6 to 12 wk (12). Organisms were partially purified by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation as previously described, washed in PBS pH 7.4, pelleted, and stored at -70°C (3). This partial purification results in *P. carinii* preparations of > 90-95% purity, with the remaining cells being host cells. An aliquot was cultured for bacteria and fungi, and preparations with bacterial (> 10 colonies) or fungal contamination were not used. Human *P. carinii* were obtained from autopsy lung specimens from patients with AIDS and *P. carinii* pneumonia, and were stored frozen before processing. Human *P. carinii* were processed in a manner identical to rat *P. carinii*, except that density gradient centrifugation was omitted (3). Density gradient centrifugation was omitted in processing human *P. carinii* because this step does not increase purity but decreases yield when using previously frozen organisms.

Antigen solubilization. Pellets of organisms were resuspended in 5-10 ml of 1 M sorbitol, 1 mM EDTA, pH 8.5, and zymolyase (ICN Biomedicals, Inc., Costa Mesa, CA), 1 mg/ml, or PBS containing 100 U/ml lyticase (Sigma Chemical Co., St. Louis, MO) (9, 13). The suspensions were incubated for 18 h at 37°C and the supernatant was clarified by centrifugation (2,000 g) for 10 min. Preparations were concentrated using a microconcentrator (Centricon-30; Amicon Corp., Danvers, MA).

Polyacrylamide gel electrophoresis and immunoblotting. SDS-PAGE was performed as previously described (2, 3, 14) based on the method of Laemmli, using a 4% stacking gel and an 8 or 10% running gel. The stacking gel buffer was 0.125 M Tris-HCl, pH 6.8, the running gel buffer was 0.475 M Tris-HCl, pH 8.8, and the electrode buffer was Tris-glycine, pH 8.3 (0.25 M Tris, 0.192 M glycine, and 0.1% SDS). Samples for SDS-PAGE were prepared by heating (100°C for 5 min) in 0.125 M Tris-HCl (pH 6.8) in the presence of 2% SDS and 5% 2-mer-

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1. Abbreviations used in this paper: BS3, bis-(sulfosuccinimidyl) substrate; gp, glycoprotein; TBS, Tris-buffered saline.

captoethanol. Gels were run at 10°C with 60 mA constant current, using bromphenol blue as a marker.

Following separation by SDS-PAGE, the gels were either stained with Coomassie blue or a silver stain (Bio-Rad Laboratories, Richmond, CA) or proteins were electrophoretically transferred to nitrocellulose and evaluated by the Western blot (immunoblot) technique (2, 3, 15). For transfer, gels were preequilibrated for 30 min in Tris-glycine (pH 8.3) in 20% methanol and transferred overnight in the same buffer at 4°C by using an electrophoretic blotting apparatus (Bio-Rad) set at 30 V. After transfer, the nitrocellulose was blocked by incubation for 60 min in 3% gelatin in Tris-buffered saline (TBS; 0.02 M Tris, 0.5 M NaCl pH 7.5), and washed in TBS containing 0.05% Tween 20. The nitrocellulose was then incubated for 2 h at room temperature with the first antibody. Antibody preparations used at this step included human serum (1:100 dilution) from patients with and without *P. carinii* pneumonia, serum from a rat immunized with rat *P. carinii*, ascites from a mouse that had been immunized with human *P. carinii* and had subsequently spontaneously developed ascites, and ascites containing MAb 6B8 (3). The first ascites reacted with multiple human *P. carinii* antigens, including the 95,000-mol wt antigen. MAb 6B8 reacts exclusively with the latter antigen (3). Following incubation, the blots were washed, incubated (2 h, room temperature) with horseradish peroxidase-conjugated goat antibody to rat IgG, mouse IgG, or human IgG (Bio-Rad), washed, and incubated with 4-chloro-1-naphthol (Bio-Rad) in the presence of 0.015% H₂O₂.

High performance liquid chromatography. Antigens were purified by HPLC using a molecular sieve column followed by an ion exchange column attached to an HPLC apparatus (Waters Chromatography Division, Millipore Corp., Milford, MA). Antigens were first separated by size using a Spherogel-TSK G2000SW column (Beckman Instruments, Inc., Fullerton, CA) under isocratic conditions at a flow rate of 0.5 ml/min using 0.1 M KPO₄, 0.2 M NaCl, pH 7.0, as the running buffer. Fractions were collected every minute, and aliquots were evaluated by SDS-PAGE and immunoblot. Fractions containing the antigen were pooled, washed with 0.01 M Tris pH 7.4, and concentrated using a Centriprep 10 concentrator (Amicon). The preparation was then applied to a Spherogel-TSK DEAE-3SW column (Beckman) and resolved by gradient chromatography using a linear gradient from 0 to 0.5 M NaCl in 0.01 M Tris, pH 7.4, at a flow rate of 1 ml/min. Fractions were collected every minute and aliquots were evaluated by SDS-PAGE and immunoblotting. The antigens were concentrated as above and washed with 40 × vol of PBS.

Deglycosylation. Antigen preparations were dissolved in 0.05 M sodium acetate, pH 5.5, 0.1% SDS, and heated (100°C) for 3 min; 20 μl (0.02 U) endoglycosidase H (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added and the sample was incubated for 24 h at 37°C; this process was repeated (16, 17).

Glycoprotein detection. Following transfer of SDS-PAGE-separated antigens to nitrocellulose, blots were blocked for 1 h at room temperature with 3% BSA in PBS. Blots were incubated for 1 h at room temperature in PBS and Con A (10 μg/ml) (Sigma) with and without 100 mM α-methylmannoside (Sigma) and washed twice (5 min each) with PBS. Blots were then incubated for 1 h with horseradish peroxidase (5 mg/ml) (Cooper Biomedical, Inc., Malvern, PA) in PBS, washed twice (5 min each) with PBS, and developed with 4-chloro-1-naphthol in the presence of 0.015% H₂O₂ (18).

Carbohydrate analysis. Purified *P. carinii* protein samples (15–50 μg) were taken to dryness, dissolved in 100 μl of 4.2 M trifluoroacetic acid, and heated at 100°C for 4 h. The acid was removed under a stream of nitrogen, and the residue taken up in an equal amount of water that was again blown to dryness. The final residue was dissolved in 100–500 μl of water and aliquots were analyzed by HPLC. The analysis was carried out by high-pH anion exchange chromatography on a BioLc Carbohydrate Analyzer (Dionex Corp., Sunnyvale, CA) employing a CarboPacPA-1 column (4.6 × 250 mm) and 10 mM NaOH as eluent. The several monosaccharides were monitored by a sensitive pulsed amperometric detector and recovered as well-separated individual peaks.

Cross-linking with bis-(sulfosuccinimidyl) substrate (BS3). Cross-linking experiments were performed using solubilized crude or partially purified antigens (20 μg) in PBS, using BS3 (0.1–1.0 mM) (Pierce Chemical Co., Rockford, IL) as the cross-linking agent (19). Preparations were incubated at room temperature for 18 h and the reaction was stopped by the addition of 0.1 vol 50 mM 2-amino-ethanol (Sigma). Results were evaluated by SDS-PAGE and immunoblotting.

IgG ELISA. 48 wells of a 96-well microtiter plate (MaxiSorp Immuno Plate; Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 μl purified human *P. carinii* antigen (0.5 μg/ml) in 0.5 M carbonate buffer, pH 9.6. The remaining wells were coated with carbonate buffer alone. The plates were washed six times with PBS containing 0.05% Tween-20, blocked for 1 h at 37°C with 200 μl 5% goat serum (Gibco Laboratories, Grand Island, NY) in PBS, and washed as before. 100-μl aliquots of human serum samples diluted (1:100) in PBS plus 5% goat serum and 0.01% Tween-20 were added to each well and plates were incubated for 1 h at 37°C. All samples were run in triplicate, and were also run in triplicate against the wells containing carbonate buffer alone. Controls on each run included a known positive sample and a known negative sample. Following washing, 100 μl of peroxidase-conjugated goat anti-human IgG (heavy and light chain specific) (Jackson Immuno Research Laboratories, Inc., West Grove, PA) diluted 1:50,000 in PBS-goat serum-Tween 20 was added to each well. After incubation at 37°C for 1 h, the plates were washed and 100 μl of 0.1% o-phenylenediamine (Sigma) in the presence of 0.003% H₂O₂ was added to each well. After 40 min at room temperature, the reaction was stopped by addition of 50 μl 20% H₂SO₄, and the absorbance was determined on a dual wavelength reader (MR 700; Dynatech Laboratories, Inc., Dynatech Corp., Chantilly, VA), using 630 nm as reference and 490 nm as the test wavelength. Optical density values for each sample were determined by subtracting the mean optical density of the triplicate wells containing no antigen from the mean of the triplicate wells containing purified protein. Optical density results were interpreted as follows: < 0.150, negative; 0.150–0.199, indeterminate; ≥ 0.200, positive.

Protein determination. Protein concentration was determined by a colorimetric assay according to the manufacturer's instructions, using BSA as the standard (BCA protein assay reagent; Pierce).

Results

Previous observations that the major rat *P. carinii* antigen, gp116, can be solubilized by treatment with zymolyase were confirmed in this study (Fig. 1) (9). The human *P. carinii* protein of 95,000 mol wt was also readily solubilized by treatment with zymolyase or lyticase, which is a similar enzyme (Fig. 1). To document that this observation was not limited to a single human *P. carinii* isolate, five additional human *P. carinii* isolates were treated in an identical manner; for each isolate a similar antigen was solubilized (gel not shown). Solubilization resulted in a small decrease in molecular weight of the rat *P. carinii*, but not human *P. carinii*, protein (Fig. 1). Following solubilization, the antigen retained its reactivity with MAb 6B8, and this antibody together with polyclonal mouse antibodies was used to identify the antigen during purification, for which a single human *P. carinii* isolate was used. Because no monoclonal antibody to the rat *P. carinii* antigen was available, polyclonal rat serum was used to identify the rat antigen.

Purification was performed in two steps. After solubilization, the antigens were partially purified by HPLC using a molecular sieve column. Both the rat and the human *P. carinii* antigen were eluted, as determined by SDS-PAGE and immunoblotting, in the void volume, and each had an apparent mol wt of > 290,000. Following ion exchange chromatography, the proteins were pure as determined by SDS-PAGE, and re-

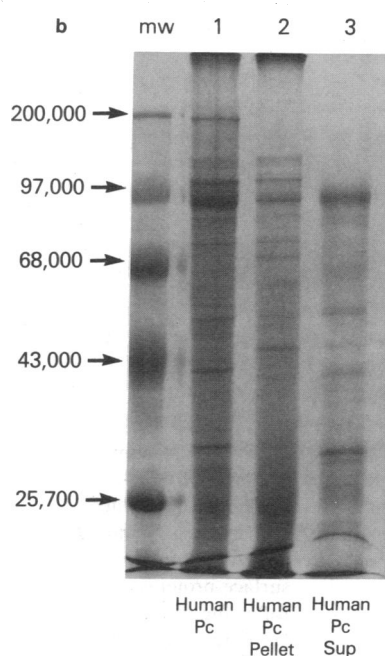
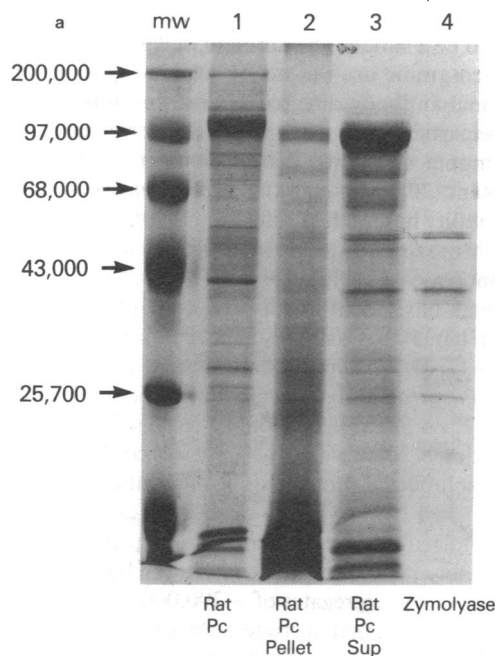


Figure 1. Coomassie blue-stained SDS-PAGE gels of rat (a) and human (b) *P. carinii* before and after solubilization of the major surface proteins with zymolyase. For both a and b, lane 1 demonstrates proteins of untreated *P. carinii* that were solubilized in SDS-PAGE buffer prior to electrophoresis; lane 2, proteins that were solubilized from the residual pellet after treatment with zymolyase; and lane 3, proteins remaining in the supernatant after zymolyase treatment. Fig. 1 a, lane 4, shows the SDS-PAGE profile of zymolyase. Molecular

weight markers indicated on the left include myosin (200,000), phosphorylase B (97,000), BSA (68,000), ovalbumin (43,000), and α -chymotrypsinogen (25,700).

mained reactive with antibody 6B8 or the polyclonal antibodies (Fig. 2).

During purification by the sizing column the native form of the human *P. carinii* surface antigen had an apparent mol wt of > 290,000, rather than 95,000 as was found under denaturing and reducing conditions on the SDS-PAGE gels. To determine whether the native antigen existed as a multimer, an attempt was made to cross-link the antigen with BS3 before denaturation and reduction. Cross-linking resulted in a decrease in the intensity of the 95,000-mol wt band, and the formation of a broad protein band of ~ 250,000–300,000 mol wt (Fig. 3). When rat gp116, which in native form elutes in a manner similar to the human *P. carinii* antigen, was cross-linked, a similar band was seen (Fig. 3). Immunoblots demonstrated reactivity of the new bands with polyclonal antibodies (Fig. 3) as well as MAb 6B8 (immunoblot not shown).

Previous investigations have demonstrated that rat *P. carinii* gp116 is a surface glycoprotein and that at least a portion of the carbohydrate moiety is susceptible to treatment with endoglycosidase H (7–11). The human *P. carinii* antigen was also found to be a glycoprotein susceptible to endoglycosidase H treatment. Treatment with this enzyme lead to a reduction in mol wt of ~ 9% for both rat and human antigens (Fig. 4). Con A bound to this antigen, and treatment with endoglycosidase H abolished this binding (Fig. 4). Despite the loss of reactivity with Con A, immunoreactivity with either polyclonal or monoclonal antibodies was retained following deglycosylation (blots not shown).

To further investigate the sugar composition of the surface antigens, carbohydrate analysis using high pH anion chromatography was performed. Two different purified human *P. carinii* antigens and a purified rat *P. carinii* antigen were analyzed by this technique. For both human *P. carinii* antigens, the major sugars were mannose, glucose, galactose, and glucosamine,

present in approximately equimolar ratios (Fig. 5). For the rat *P. carinii* antigen, mannose and glucose were also present in approximately equimolar ratios, but glucosamine and galactose were each present at one-fifth the concentration of mannose or glucose (Fig. 5). Because sialic acid is destroyed by trifluoroacetic acid, the sialic acid content of the glycoproteins could not be determined by this procedure.

To evaluate the immune response of humans to gp95, an ELISA was developed, as described in the methods, for detection of IgG antibodies to the purified antigen. Optimal conditions were determined by preliminary experiments. To validate the reliability of the ELISA, 31 samples were evaluated in parallel ELISA and immunoblot assays (Fig. 6). A sample was considered positive by immunoblot if any band of 95,000 mol wt was seen. In 25 samples, there was agreement between ELISA and immunoblot results; among the remaining 6 samples, all of which were indeterminate by ELISA, 2 were positive and 4 were negative by immunoblot. The patient from whom the human *P. carinii* isolate used in these studies was obtained demonstrated reactivity with gp95 by both ELISA and immunoblot. Additional sera from healthy and immunosuppressed humans were then evaluated by ELISA, and results are summarized in Table I. Among 27 infants hospitalized for diarrhea, one value was indeterminate, and the remaining 26 were negative. No healthy adult laboratory worker had detectable antibodies against the gp95 antigen. Among AIDS and non-AIDS immunocompromised patients with no history of *P. carinii* pneumonia, only 1 of 16 sera were positive by ELISA. Among 30 AIDS and non-AIDS patients with a history of *P. carinii* pneumonia, 10 patients had at least 1 sample positive for antibodies to gp95 by ELISA, 7 patients had indeterminate samples, and 13 had negative samples. When sera obtained only within 3 wk of an episode of *P. carinii* pneumonia were evaluated, 7 samples were positive, 5 were indeterminate, and 12

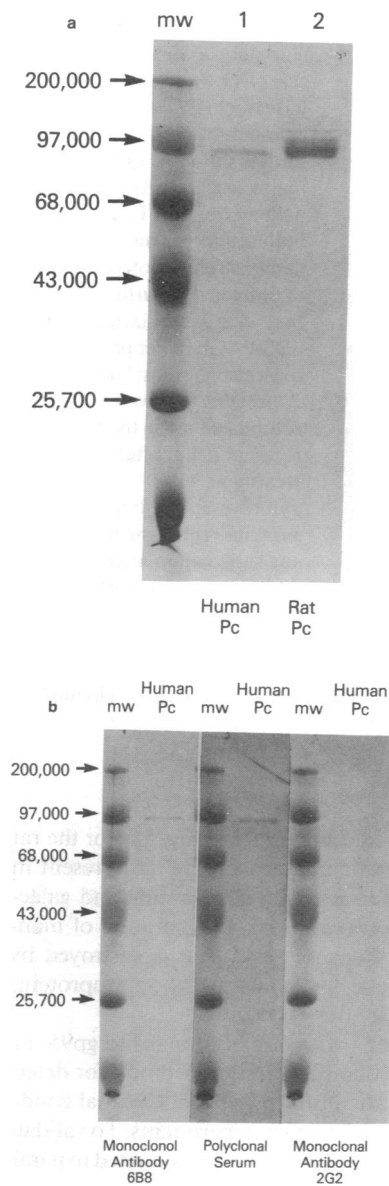


Figure 2. (a) Coomassie blue-stained SDS-PAGE gel of purified human (lane 1) and rat (lane 2) *P. carinii* surface proteins following molecular sieve and ion-exchange chromatography. (b) Immunoblot analysis of human *P. carinii* gp95 after purification. Panels 1 and 2 show reactivity of the purified antigen with MAb 6B8 and polyclonal antibodies, respectively. For panel 3, which shows no reactivity, the blot was incubated with MAb 2G2, which is specific for a 65,000-mol wt antigen of human *P. carinii*. Molecular weight markers indicated on the left are the same as for Fig. 1.

were negative. Among six AIDS patients with a history of *P. carinii* pneumonia for whom serial serum samples were available before and after the episode of pneumonia, no consistent pattern of reactivity was seen. Some patients developed antibodies to gp95 before or concomitant with the development of clinical disease, others lost antibodies before development of overt pneumonia, and still others never developed detectable antibodies despite multiple episodes (Fig. 7).

Discussion

Studies of the epidemiology and immunology of *P. carinii* infection in humans have been hampered by an inability to obtain purified human *P. carinii* antigens in sufficient quantities. Although the immunoblot technique has recently been successfully used by our group as well as other investigators to address these issues (2-6), purified antigens would greatly facilitate such studies. By SDS-PAGE and immunoblot, a major rat *P. carinii* antigen of 110,000-120,000 mol wt (gp116) has been

identified by numerous investigators (2, 7-11). This antigen has been found to be a surface glycoprotein in which mannose and *N*-acetylglucosamine are the major sugar residues (7, 9, 11). A monoclonal antibody directed against this antigen has been found to be partially protective in a rat and ferret model of *P. carinii* pneumonia, suggesting that this antigen is immunologically important (20). Although this protein has been purified, lack of solubility has made purification of large quantities difficult (7, 11). More recently, other investigators have demonstrated that zymolyase can solubilize this antigen (9).

We and others have identified a predominant protein of human *P. carinii* (gp95) that is slightly smaller than the predominant rat antigen (2, 3). We hypothesized that the predominant rat and human *P. carinii* antigens were homologous, and thus that the human *P. carinii* antigen would also be solubilized by zymolyase or lyticase. After preliminary studies confirmed this hypothesis, the solubilized proteins of both rat and human *P. carinii* were purified by a two-step procedure and partially characterized. Molecular sieve and cross-linking studies demonstrated that before reduction and denaturation both proteins existed as multimers or aggregates of > 290,000 mol wt. Radding et al. similarly found that in crude surface protein preparations the major surface protein of rat *P. carinii* had an apparent mol wt of > 1,000,000 (7). The lower molecular weight we observed following cross-linking (250,000-300,000 mol wt) may be due to inefficient cross-linking or to different solubilization methods.

Lectin studies have previously shown that the predominant antigens of both rat and human *P. carinii* are glycoproteins that contain mannose and *N*-acetylglucosamine as the major carbohydrates (9, 10, 21). This study confirms that these antigens are glycoproteins that are susceptible to endoglycosidase H; following treatment with this enzyme, there was an ~ 9% decrease in the molecular weight of both glycoproteins, similar to results previously reported for the rat antigen. Identical results were seen with endoglycosidase F (results not shown). Radding et al. previously found, however, that such deglycosylation was incomplete, and following chemical deglycosylation of gp116 with trifluoromethanesulfonic acid, the major core protein had a mol wt of 68,000 (7). Attempts to deglycosylate the human *P. carinii* gp95 with trifluoromethanesulfonic acid were unsuccessful in our study. Susceptibility to endoglycosidase H and F demonstrate that both rat and human *P. carinii* glycoproteins contain high-mannose or possibly mixed oligosaccharides that are N-linked to the core protein. Since reactivity with Con A was eliminated by treatment of the proteins with endoglycosidase H (Fig. 4), any carbohydrate moiety remaining after such treatment is either not reactive with or inaccessible to Con A.

Carbohydrate analysis of rat and human *P. carinii* glycoproteins demonstrated distinct differences. Whereas in two human *P. carinii* isolates mannose, glucose, galactose, and glucosamine were present in equimolar concentrations, in the rat *P. carinii* glycoprotein glucose and mannose were present in five-fold higher concentrations than the two other carbohydrates. Tanabe et al., using less sensitive techniques, previously found mannose to be the predominant carbohydrate of the major rat *P. carinii* glycoprotein (11). The occurrence in our study of sugars other than mannose in high molar ratios suggests that in addition to the high-mannose residues, mixed or complex N-linked or O-linked oligosaccharides also are present. The presence of glucose in the glycoproteins is unusual, and raises the question of an artifactual result. However, no glucose-contain-

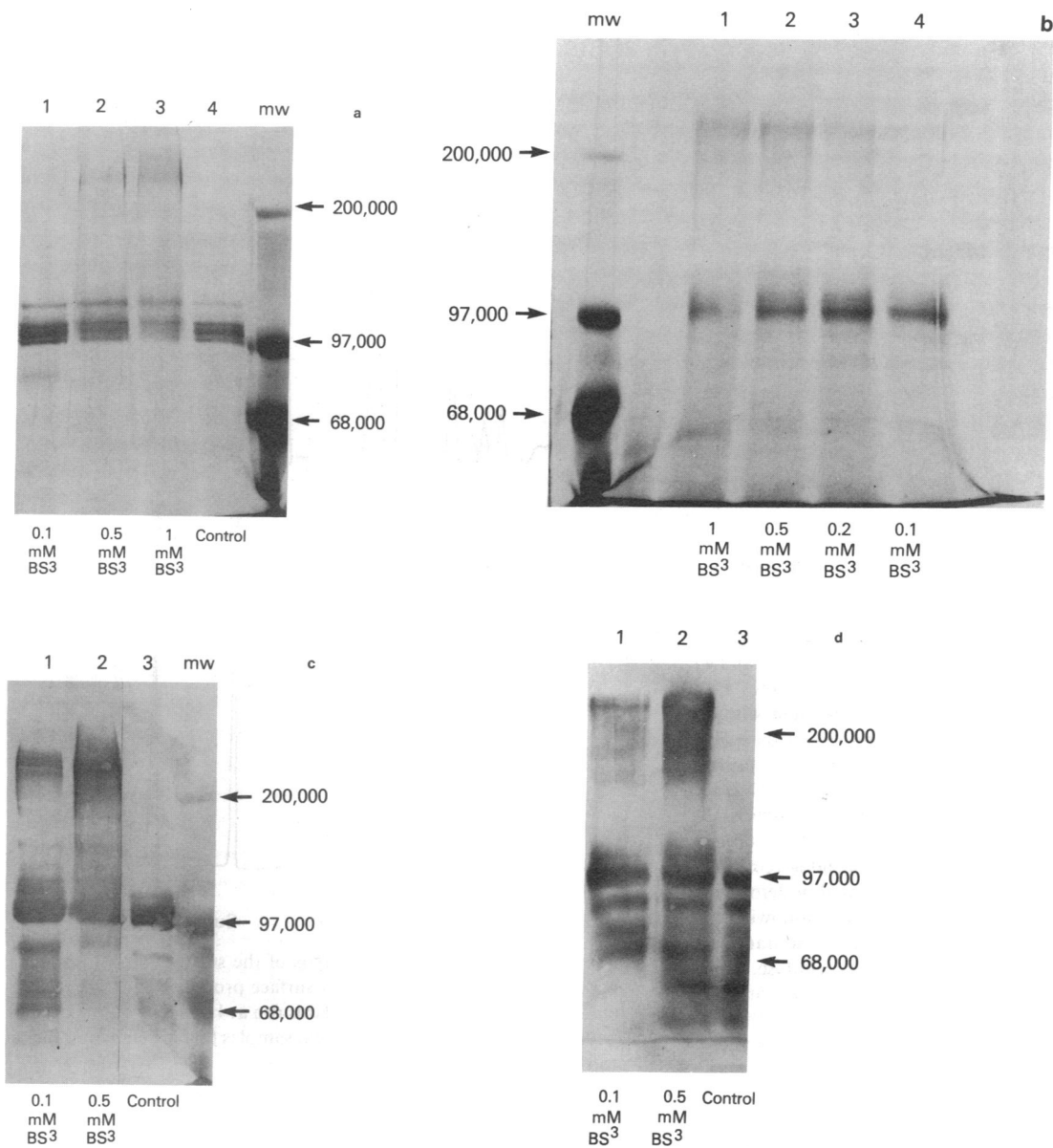


Figure 3. (a) and (b) Demonstration of the appearance of a new, high molecular weight band following cross-linking of rat (a) and human (b) *P. carinii* proteins by BS3. Partially purified antigen preparations were cross-linked with BS3; proteins were then separated by SDS-PAGE, and the gel was stained with Coomassie blue. The concentration of BS3 is indicated at the bottom of the figures. As the concentration of BS3 was increased, the intensity of the original band decreased, and the intensity of the new band of 250,000–300,000 increased. (c) and (d) Immunoblots demonstrating the immunoreactivity of the new, high molecular weight bands of rat (c) and human (d) *P. carinii*. The concentrations of BS3 are indicated at the bottom.

ing reagent was used during the purification, suggesting that the observation is real.

Susceptibility of *P. carinii* to zymolyase and the presence of glycoproteins containing high-mannose residues suggests a structural similarity to *Saccharomyces cerevisiae* (22). Zymolyase contains two enzymatic activities, a β -1,3 glucanase as well as a protease, both of which are necessary for lysis of yeast (13); this study cannot determine which of these activities is important in solubilizing the surface proteins. Previous investigators have suggested that β -1,3 glucan is present in the cell wall of *P. carinii*, and recent studies indicate that *P. carinii* may contain β -glucan synthase activity, further suggesting a similarity to yeast (9, 23). It is possible that the glucose detected in the

purified glycoprotein preparations is derived from the glucan matrix.

To evaluate the immune response to human *P. carinii* gp95, an ELISA using purified protein was developed. Previous studies using immunofluorescence or the immunoblot technique have shown that most adults have antibodies to human *P. carinii* (2, 24–28). We and others have found that despite the abundance of gp95 (as evaluated by Coomassie blue stain), it does not appear by immunoblot to be an immunodominant protein (2, 4, 29). Rather, most humans have antibodies to a broad band of 35,000–45,000 mol wt that cannot be identified on Coomassie blue-stained gels. This study supports these observations. Only one individual without a history of *P. carinii*

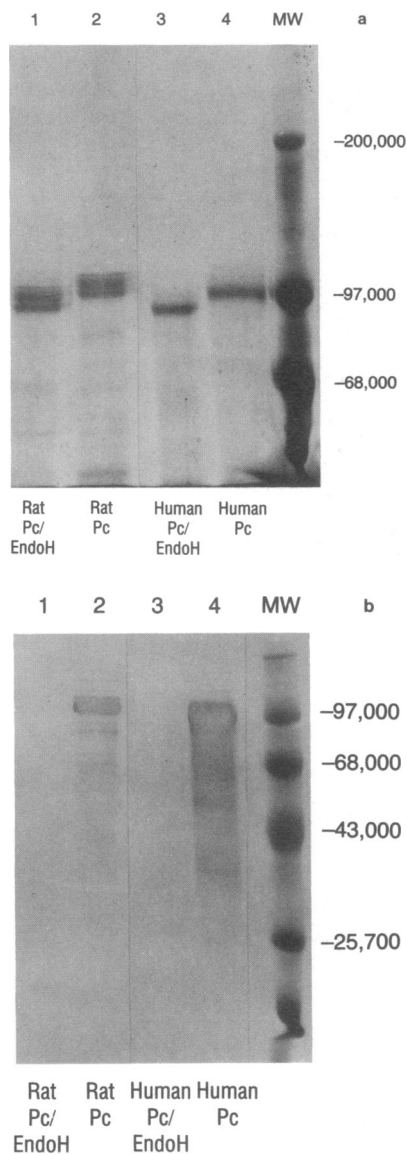


Figure 4. (a) Silver stained SDS-PAGE gel of rat and human *P. carinii* surface antigens before (lanes 2 and 4) and after (lanes 1 and 3) treatment with endoglycosidase H. A reduction in molecular weight of ~ 9% is seen for both proteins. (b) Reactivity of untreated and endoglycosidase-treated proteins with Con A following SDS-PAGE and transfer to nitrocellulose. There is loss of reactivity with Con A for both proteins following enzyme treatment.

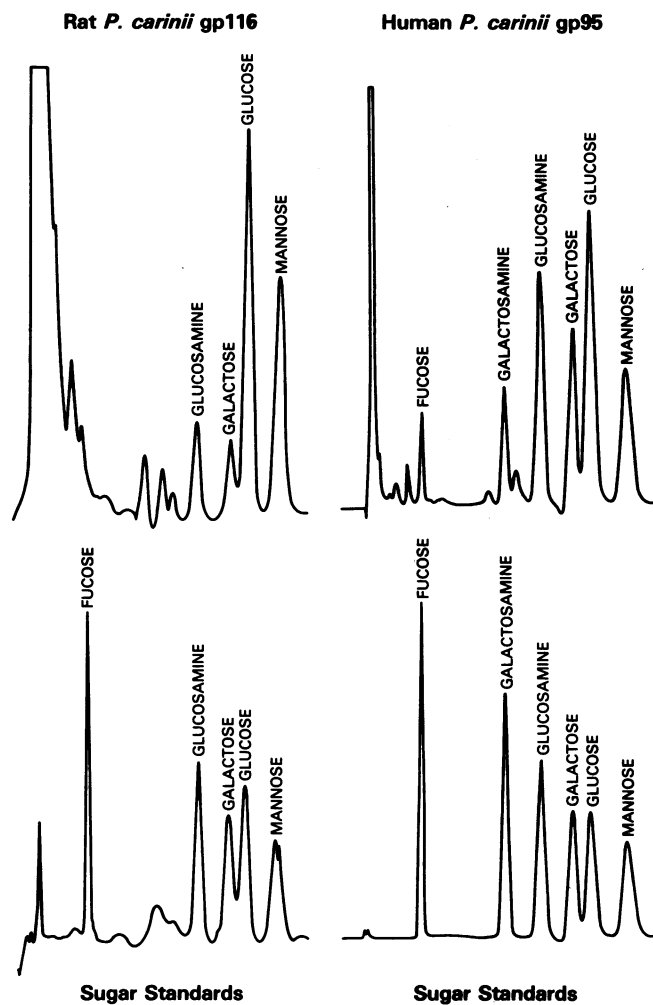


Figure 5. Chromatographic analysis of the sugar composition of purified rat and human *P. carinii* surface proteins. The two bottom chromatograms are of sugar standards run at 1-nmol levels in parallel with the rat and the human *P. carinii* samples that are shown in the top chromatograms.

Table I. gp95 ELISA Results

Patient population	History of <i>P. carinii</i> pneumonia	Positive n (%)	Indeterminate n (%)	Negative n (%)	Total
Infants	No	0 (0)	1 (4)	26 (96)	27
	Healthy laboratory workers	0 (0)	0 (0)	8 (100)	8
	Non-AIDS, immunosuppressed	0 (0)	1 (33)	2 (67)	3
AIDS	Yes	4 (33)	3 (25)	5 (42)	12
	Yes, within 3 wk	4 (40)	2 (20)	4 (40)	10
	No	1 (20)	1 (20)	3 (60)	5
	Yes	6 (33)	4 (22)	8 (45)	18
	Yes, within 3 wk	3 (21)	3 (21)	8 (58)	14

Detection of IgG to gp95 by ELISA for the indicated patient populations. Serum (diluted 1:100) was evaluated in triplicate, and the mean of the results was used to categorize reactivity. Patients were categorized as having a history of *P. carinii* pneumonia if they had any history of this infection. Patients with *P. carinii* pneumonia within 3 wk of obtaining the evaluated serum sample are a subpopulation of all patients with *P. carinii* pneumonia.

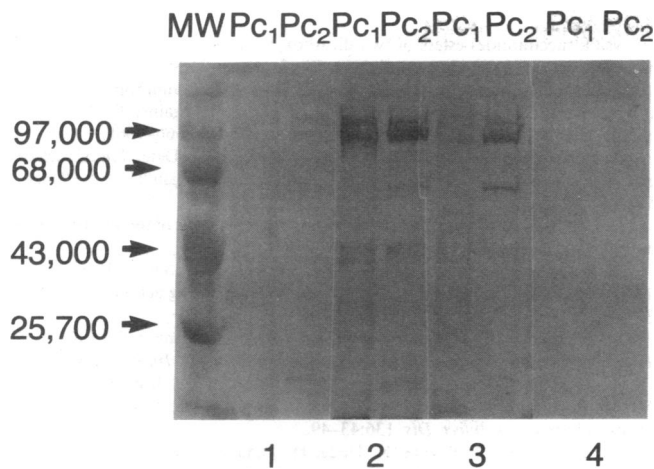


Figure 6. Immunoblots of two partly purified human *P. carinii* samples that were reacted with four sera (1:100 dilution) obtained from the following patients: 1, non-AIDS immunosuppressed patient with *P. carinii* pneumonia; 2 and 3, AIDS patients with *P. carinii* pneumonia; 4, healthy laboratory worker. The first three serum samples, which react with gp95 by immunoblot, were positive in the gp95 ELISA, and the fourth sample was negative by both immunoblot and ELISA.

pneumonia (a patient with HIV infection and a history of Kaposi's sarcoma) of 43 tested had clearly detectable antibodies to gp95, and three had indeterminate results. 10 of 30 immunocompromised patients with a history of *P. carinii* pneumonia had detectable antibodies, and an additional 7 had indeterminate results. In patients with *P. carinii* pneumonia for whom serial samples were available, variable responses were seen. Some patients developed no antibody response despite multiple episodes of pneumonia, others developed antibodies after clinical disease, and yet others developed detectable antibodies before diagnosis of *P. carinii* pneumonia (Fig. 7). Larger studies evaluating the usefulness of antibody detection are needed, but the current study suggests that in a subpopulation of patients clinical *P. carinii* pneumonia may be heralded by the development of such antibodies. Whether such antibodies are predictive of a favorable outcome cannot be evaluated by this study, but as previously noted, monoclonal antibodies to the analogous ferret and rat antigen may be protective (20).

It is unclear why so few humans have antibodies to gp95. It is possible that antigenic variability, with little cross-reactivity, occurs among different human *P. carinii* strains. However, at least one epitope, that identified by antibody 6B8, reacts with all isolates tested to date, and human sera that react by immunoblot with one isolate also react with other isolates (3). Alternatively, the antigen may not be exposed to the host immune system, although immunofluorescent studies demonstrate surface reactivity with 6B8. It is also possible that the antigen is structurally related to host antigens resulting in tolerance.

The availability of purified *P. carinii* surface antigens will allow more precise investigations of the function of these proteins and the host immune response to them. In preliminary studies, no blastogenic response to gp95 by peripheral blood mononuclear cells obtained from normal hosts was seen. Previous investigators have demonstrated blastogenic responses to *P. carinii* in healthy adults, using crude antigen preparations (30–32). Since the antigens used in those studies were

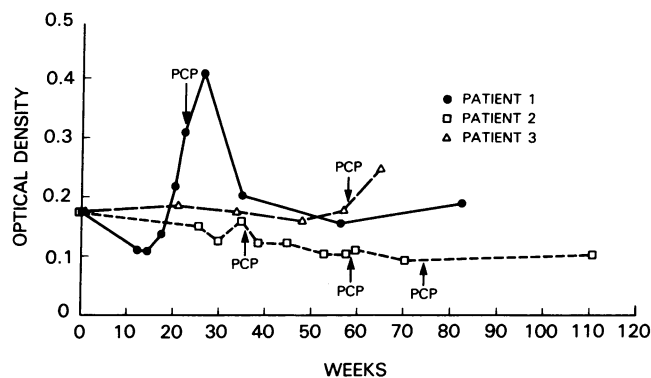


Figure 7. Changes in ELISA values with time in three patients with *P. carinii* pneumonia. Serial samples were available for these patients before and after episodes of *P. carinii* pneumonia, which are indicated by the arrows. Weeks after obtaining the first serum sample are shown along the bottom, and optical density is shown along the left. Patient 1 had an increase in optical density prior to the development of *P. carinii* pneumonia. Patient 2 had no change in optical density despite three episodes of *P. carinii* pneumonia, and patient 3 had an increase after developing pneumonia.

not purified or well characterized, it is possible that the blastogenic responses were nonspecific. Alternatively, the blastogenic responses may not have been to the major surface antigen, but to other, more immunogenic, antigens. Further studies to evaluate blastogenic responses in patients at risk for *P. carinii* pneumonia may help explain the role of T cell-mediated immunity in protecting against this infection.

The role of the major surface glycoprotein of *P. carinii* is unknown; speculation has centered on mediating attachment to host cells or on a role in evasion of host defense mechanisms (7, 11). Recent studies, for example, have suggested that *P. carinii* attachment to tissue culture cells is mediated by fibronectin, and that rat *P. carinii* gp116 is the receptor for this attachment (33, 34). Whether the human *P. carinii* antigen interacts with fibronectin or with other cell receptors, such as the recently described mannose-binding protein, remains to be determined by further investigations (35, 36). Nonetheless, the distinctive carbohydrate profiles of *P. carinii* isolated from different species and the previously documented antigenic differences between rat and human isolates suggest that this antigen plays a role in determining host specificity or host-organism interactions.

In summary, we have purified and partially characterized major surface antigens of rat and human *P. carinii*. Extension of these studies will help us to better understand this pathogen, and ultimately may lead to improved management of *P. carinii* pneumonia.

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