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Localization of Host Immunoglobulin G to the Surface of *Pneumocystis carinii*†

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In immunoblotting studies of *Pneumocystis carinii* surface proteins, we found that a secondary antibody, anti-human immunoglobulin G (IgG), recognized a 52-kilodalton (kDa) band in homogenates of *P. carinii* purified from human autopsy lungs and bronchoalveolar lavage fluids, even when serum as a source of primary antibody was omitted. The electrophoretic mobility of the 52-kDa band is identical to that of IgG heavy chains. In addition to affinity-purified, anti-human IgG, monoclonal antibodies specific for the Fab and Fc regions of human IgG recognized the 52-kDa band. To determine whether the 52-kDa band represents IgG bound to the surface of *P. carinii*, we treated intact organisms with Triton X-100 and acid in order to elute immunoglobulin from the surface of *P. carinii*. After purification over a protein G column, the eluate comigrated with human IgG, was recognized by anti-IgG, and bound to discrete bands with molecular sizes of 65 to 70, 60, 50, and 35 kDa in purified, rat-derived *P. carinii*. To confirm the presence of human IgG on the surface of *P. carinii*, we performed immunocytochemical and immunoelectron microscopic studies. Staining of intact *P. carinii* aggregates by anti-human IgG was pronounced and was abolished by acid treatment. IgA was also present. Ultrastructural studies showed the presence of IgG on the cyst wall and on fine membranous structures and vesicles adjacent to cysts. We conclude that the surface of *P. carinii* is coated with human IgG. The close association of human IgG with *P. carinii* may have implications for the pathogenesis of *P. carinii* pneumonia in acquired immunodeficiency syndrome.

Pneumocystis carinii pneumonia is a major cause of morbidity and mortality in patients with acquired immunodeficiency syndrome (AIDS) and may occur in up to 80% of individuals with AIDS (16). The reasons for the high prevalence of *P. carinii* in AIDS patients are unclear. There is evidence to suggest that *P. carinii* is ubiquitous and that most people have serologic evidence of exposure to *P. carinii* (13, 15, 17). It remains unclear whether the presence of *P. carinii* signifies the reactivation of latent organisms or de novo acquisition. The mechanisms whereby *P. carinii* is either reactivated or repetitively acquired and subsequently proliferates in an immunocompromised setting are unknown.

P. carinii appears to be uniquely suited to take advantage of the human immunodeficiency virus (HIV)-infected alveolar microenvironment. This environment, as assessed by analyses of bronchoalveolar lavage fluid from patients, contains alveolar macrophages and suppressor-cytotoxic lymphocytes as the principal cellular constituents and increased immunoglobulin G (IgG) and IgA levels and IgG-releasing cells compared with controls (19, 25). Many of the macrophages are infected with HIV (19), and many of the lymphocytes are functionally HIV-specific cytotoxic T lymphocytes (18). It is not clear how much of the cellular and humoral response is specifically directed against *P. carinii*. It is reasonable to hypothesize that proliferation of *P. carinii* proceeds in the absence of immune containment because the host either fails to respond or responds ineffectively.

We have found that *P. carinii* obtained from patients with AIDS, in common with *P. carinii* obtained from children with plasma cell pneumonitis (5) and adults without AIDS (14), is coated with host immunoglobulins. This suggests that in AIDS patients, there is at least a partial host response to *P. carinii*. In addition, since *P. carinii* is not readily culturable in vitro, the study of *P. carinii* antigens purified from a host lung requires the separation of host immunoglobulins from organisms.

MATERIALS AND METHODS

Source of antibodies. We obtained the following from Calbiochem, La Jolla, Calif.: human IgG Fc fragments, IgA, IgM, two monoclonal antibodies to the Fc fragment of human IgG, and one monoclonal antibody to an Fab fragment of human IgG. The following were obtained from Boehringer-Mannheim, Indianapolis, Ind.: alkaline phosphatase or horseradish peroxidase conjugated to goat anti-human immunoglobulins, anti-human IgG, anti-human IgM, anti-rat IgG, anti-mouse IgG, and anti-mouse IgG $F(ab')_2$. From Vector Laboratories, Burlingame, Calif., we obtained biotinylated anti-human IgG, anti-human IgA, anti-human IgM, anti-mouse IgG, avidin-biotinylated macromolecular complexes conjugated to horseradish peroxidase and to alkaline phosphatase, and alkaline phosphatase substrate. Anti-human IgG conjugated to 10-nm colloidal gold was obtained from Jansen Life Sciences Products, Piscataway, N.J. Diaminobenzidine tetrahydrochloride was from Sigma Diagnostics, St. Louis, Mo.

Source of *P. carinii*. Human lung material with and without *P. carinii* was obtained at autopsy and stored at −70°C. To obtain purified *P. carinii*, fragments (1 by 1 cm) were thawed. Alveolar contents were expressed from lung parenchyma with a stomacher (6), and then the *P. carinii* was separated from host cells by density gradient centrifugation with Ficoll-Paque (Pharmacia, Piscataway, N.J.) (1). The interface between the supernatant and the Ficoll-Paque, which contains the majority of *P. carinii* organisms, was collected. Portions (5 μl) of material were stained with

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Diff-Quik (American Scientific Products, Chicago, Ill.) and microscopically examined to assess the adequacy of the purification. A preparation was considered adequate and used in further studies if it contained less than 1% host cells and contained at least 10 aggregates of *P. carinii* per low power field. Rat lungs containing *P. carinii* were processed in an identical manner. The immunocytochemical and immunoelectron microscopic studies were done, using human-derived bronchoalevolar lavage fluid containing *P. carinii* that either was fresh or had been stored at −70°C in 20% glycerol in phosphate-buffered saline, pH 7.4.

SDS-PAGE and immunoblotting. Sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (11), using a miniprotean dual slab cell and mini-trans-blot electrophoretic transfer cell (Bio-Rad, Richmond, Calif.). The stacking gel was 4% acrylamide–0.125 M Tris, pH 6.8, and the separating gel was 7.5% acrylamide–0.375 M Tris, pH 8.8. Protein concentrations were determined by the Pierce protein assay (Pierce Chemical Co., Rockford, Ill.). Samples were solubilized in an SDS-reducing buffer consisting of 0.0625 M Tris hydrochloride (pH 6.8), 10% (vol/vol) glycerol, 2% SDS, 5% 2-mercaptoethanol, and bromophenol blue and boiled for 5 min. Protein (10 to 20 μg) was loaded into each lane and run along with prestained molecular weight markers (Sigma Corp., St. Louis, Mo., and Bio-Rad). Gels were either stained with Coomassie stain or electroblotted onto nitrocellulose (21) after equilibration in 25 mM Tris–192 mM glycine–20% (vol/vol) methanol, pH 8.3. Blots were first exposed to 10% Carnation milk powder in phosphate-buffered saline (PBS), pH 7.4, for 1 h to saturate nonspecific binding sites. Blots were then exposed overnight to monoclonal antibodies diluted 1:500 or 1:1000 in Tris-buffered saline with 0.1% Tween 20, pH 7.5. Murine ascites or isotype-matched irrelevant monoclonal antibodies were included as controls in every experiment. After incubation with the primary antibody, blots were sequentially exposed, with in-between washes of Tris-buffered saline with 0.1% Tween 20, either to biotinylated anti-mouse IgG for 1 h, enzyme tagged (alkaline phosphatase or peroxidase) avidin-biotin conjugate for 1 h, and then the appropriate enzyme substrate; or to enzyme-tagged anti-mouse IgG for 1 h and then the appropriate enzyme substrate. In some experiments, blots were not exposed to a primary antibody but were directly exposed to enzyme-tagged anti-IgG and then the appropriate enzyme substrate.

Elution of immunoglobulins from the surface of *P. carinii*. Human-derived organisms that had been purified by density gradient centrifugation were divided into equal volumes of 100 μl and combined at room temperature with an equal volume of either (i) PBS (pH 7.4) for 30 min, (ii) 0.1% Triton X-100 in PBS (pH 7.4) for 10 min, (iii) 0.1 M glycine-HCl with 0.5 M glucose (referred to as glycine-HCl) (pH 2.9) (7) for 10 min, (iv) glycine-HCl for 1 h, or (v) 0.1% Triton X-100 for 10 min followed by glycine-HCl for 10 min. After the designated time, the material was centrifuged for 1 min in a Fisher microcentrifuge (model 235B), and the supernates were separated from the precipitates. The supernates were adjusted to pH 7.4 with 1 M Tris (pH 8.2) and dialyzed against PBS. The precipitates were washed and suspended in PBS. The contents of the precipitates and supernates were then evaluated by SDS-PAGE and immunoblotting with anti-human IgG. Selected supernates were added to a protein G column (Pharmacia). After the nonadherent fraction was collected, the retained fraction was eluted from the column with glycine-HCl. The fractions containing proteins, as determined by the Pierce protein assay, were pooled and adjusted in PBS (pH 7.4) to a concentration of 0.25 to 0.5 μg/μl with Centricon microconcentrators (Amicon, Danvers, Mass.).

Immunocytochemistry. Immunocytochemistry was performed, using bronchoalveolar lavage sediment as previously described (3). Bronchoalveolar lavage sediment (10 μl) containing *P. carinii* was applied to microscopic slides. The slides were air dried and left unfixed or were fixed either in acetone or methanol for 10 min. After exposure to normal goat serum (20 μl in 5 ml of PBS [pH 7.4]), the slides were incubated for 1 h with a 1:100 dilution of conjugated anti-human IgG, IgM, or IgA. After a PBS rinse, the slides were exposed to enzyme substrate and counterstained with hematoxylin solution Gill no. 2 (Sigma Diagnostics). In some experiments, slides were exposed to glycine-HCl buffer with 0.5 M glucose (pH 2.9) for varying lengths of time (15 min, 1 h, 4 h, or 12 h) after fixation to elute off adherent immunoglobulin (7). After exposure to glycine-HCl buffer, slides were rinsed in PBS (pH 7.4) for 20 min with two changes of PBS and then exposed to normal goat serum followed by anti-human immunoglobulin as described above. Additional immunocytochemical experiments were done in an identical manner with acetone-fixed monolayers of irradiated A549 cells to which human-derived *P. carinii* had been added (2).

Immunoelectron microscopy. Immunoelectron microscopy was done with thawed bronchoalveolar lavage fluid containing *P. carinii* that had been stored at −70°C in 20% glycerol in PBS. Bronchoalveolar lavage fluid sediment (from a specimen which contained copious amounts of *P. carinii* aggregates as assessed by Diff-Quik staining) was divided into two aliquots of 300 μl each. A 300-μl portion of glycine-HCl buffer with 0.5 M glucose, pH 2.9, was added to one of the aliquots. After 10 min, both aliquots were suspended in PBS, washed, and suspended in 100 μl of PBS. Anti-human IgG-colloidal gold (20 μl) was added to each. After 1 h, the specimens were washed three times in PBS and suspended for 2 h in bicarbonate buffer (pH 7.4) containing 2.7% glutaraldehyde and 0.8% paraformaldehyde. The specimens were then postfixed in 2% reduced osmium, dehydrated in ethanol, and embedded in LX-112 plastic (Ladd, Burlington, Vt.). Thin sections were cut with a Sorvall microtome MT2B and placed on Formvar-coated nickel grids. They were stained for 5 min with Reynolds lead citrate and photographed on a Philips 201 electron microscope.

RESULTS

Immunoblotting studies. We initially noted difficulty in the interpretation of immunoblots in which human sera were blotted against purified, human-derived *P. carinii*. In control blots in which serum was omitted, there was consistent reactivity of the secondary antibody, anti-human IgG, to a 52-kilodalton (kDa) band. This 52-kDa band was present in *P. carinii* purified from lungs obtained at autopsies of two AIDS patients and in 8/10 bronchial lavage fluid sediments with *P. carinii*. It was also present in the autopsy-obtained lung of an HIV-seronegative individual who died of myocardial infarction without pulmonary infection and in 1/5 bronchial lavage fluid sediments without *P. carinii*. The molecular sizes of human IgG heavy chains are as follows: IgG1, 51 kDa; IgG2, 51 kDa; IgG3, 60 kDa; and IgG4, 51 kDa (20). To investigate the possibility that the 52-kDa band present in purified *P. carinii* represented host IgG heavy chains, we reacted blots containing purified *P. carinii* with monoclonal

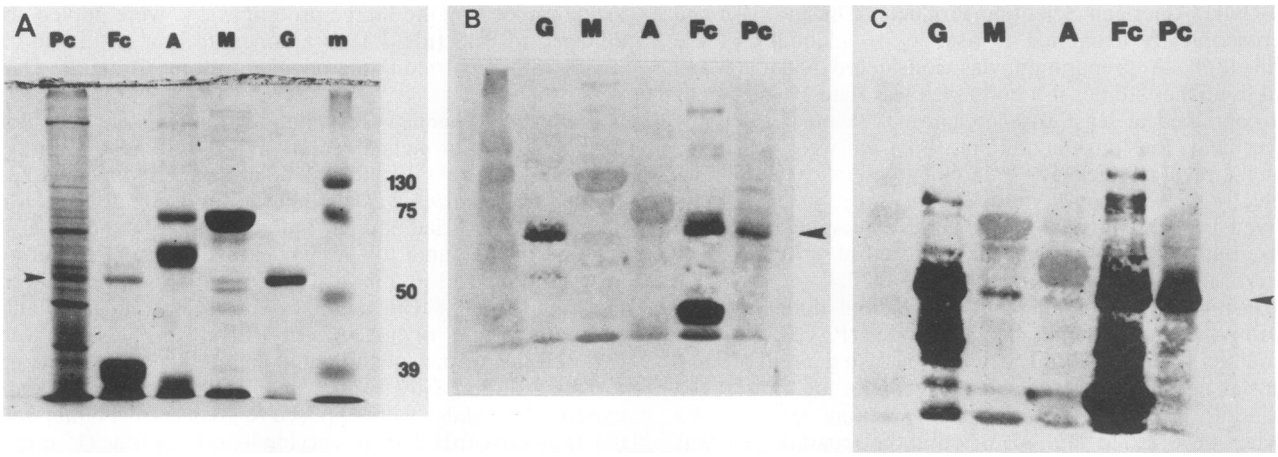

FIG. 1. Recognition of 52-kDa band in purified *P. carinii* by monoclonal antibodies to Fab and Fc regions of human IgG. The arrowhead denotes the 52-kDa band. (A) Coomassie stain of gel; (B) Western blot with anti-Fab as primary antibody; (C) Western blot with anti-Fc as primary antibody. Lanes: Pc, purified human-derived *P. carinii*, 20 μg; Fc, Fc fragment of human IgG, 10 μg; A, human colostrum IgA, 10 μg; M, human IgM, 10 μg; G, human IgG, 10 μg; m, molecular weight markers. Numbers on right indicate molecular sizes in kDa.

antibodies specific for the Fab and Fc regions of human IgG. A monoclonal antibody to the Fab region of human IgG (Calbiochem 411440) and one of two monoclonal antibodies to the Fc region of human IgG (Calbiochem 411431) recognized the 52-kDa band in preparations of purified *P. carinii* (Fig. 1). Anti-rat IgG, anti-mouse IgG, and anti-mouse IgG $F(ab')_2$ did not recognize any material in purified, human-derived *P. carinii* (data not shown). Anti-human IgM recognized a 70-kDa band, and anti-human IgA recognized two bands with approximate molecular sizes of 55 and 60 kDa in purified, human-derived *P. carinii* (results not shown).

Removal of immunoglobulins from the surface of *P. carinii*. In order to determine whether the 52-kDa band represents IgG bound to the surface of *P. carinii*, we attempted to elute and purify immunoglobulins from intact organisms. Because the optimal conditions for elution of immunoglobulins from the surface of *P. carinii* were not known, several conditions were evaluated, along with a PBS buffer control. These included Triton X-100 for 10 min, glycine-HCl for 10 min, glycine-HCl for 60 min, and Triton X-100 for 10 min followed by glycine-HCl for 10 min. The resultant precipitates and supernates were evaluated by SDS-PAGE and immunoblotting with anti-human IgG.

Many bands were visible by Coomassie staining in both the precipitates and the supernates. Of the precipitates, the 52-kDa band was visible by Coomassie staining only in the PBS-treated precipitate (Fig. 2A). Anti-human IgG bound a 52-kDa band strongly in the PBS-treated precipitate, less strongly in the precipitate exposed to glycine-HCl for 10 min, weakly in the precipitates exposed to Triton X-100 for 10 min and glycine-HCl for 1 h, and faintly in the precipitate exposed to Triton X-100 followed by glycine-HCl (Fig. 2B). Two bands with approximate molecular sizes of 42 and 45 kDa were also recognized by anti-human IgG strongly in the PBS control, weakly after exposure to Triton X-100 or glycine-HCl, and not at all after exposure to both Triton X-100 and glycine-HCl (Fig. 2B).

The 52-kDa band was visible by Coomassie staining in the supernates treated with Triton X-100 alone or in combination with glycine-HCl (Fig. 3A). All of the supernates (except for the PBS control, which reacted weakly) contained the 52-kDa band that was strongly recognized by anti-IgG (Fig. 3B). All of the supernates except for the PBS control contained the 45-kDa band recognized by anti-human IgG. The glycine-HCl (60 min) and Triton X-100 supernates also contained a 42-kDa band recognized by anti-human IgG.

The supernates produced after exposure to Triton X-100 for 10 min and glycine-HCl for 60 min were passed onto a protein G column to purify the IgG (Fig. 4). The nonadherent fraction did not contain bands by Coomassie staining or by reactivity with anti-human IgG. In contrast, the adherent fraction that was eluted from the protein G column with glycine-HCl contained a prominent 52-kDa band by Coomassie staining and by reactivity with anti-human IgG. Several additional bands were present as well. The molecular sizes of the major additional bands were approximately 140, 100, and 25-kDa and correspond to known molecular sizes of intact and fragmented IgG (20). The IgG purified from the eluate recognized discrete bands in electrophoretically separated, purified, rat-derived *P. carinii* with molecular sizes of 65 to 70, 60, 50, and 35 kDa (Fig. 5).

Immunocytochemical studies. The above studies strongly suggested that the surface of *P. carinii* contained adherent host immunoglobulin molecules but did not rule out the possibility that immunoglobulins in the alveolar space or lavage fluid were being purified along with *P. carinii*. Therefore, immunocytochemical studies were done to see if immunoglobulins could be localized to the surface of *P. carinii*. With the use of a black substrate, anti-human IgG but not anti-rat IgG gave a pronounced black colorimetric reaction to aggregates of *P. carinii* at the light microscopic level (Fig. 6) in 4/4 bronchoalveolar lavage fluid sediments. The reaction was independent of fixation method. There was variability among the four cases, with two cases staining more intensely than the other two. The specificity was sufficient to allow rapid and easy detection of *P. carinii* aggregates at low power (100×). The elution of immunoglobulins by exposure to glycine-HCl buffer (pH 2.9) prior to immunolabeling of acetone or methanol-fixed material resulted in the loss of the colorimetric reaction. IgA was also demonstrable on the surface of *P. carinii* by immunostaining with anti-human IgA but to a considerably lesser extent than was IgG. IgM was not present. Irradiated A549 cell monolayers to which human-derived *P. carinii* was added and which subsequently were incubated at 37°C for 3 or 31 h were reacted with anti-human IgG. The *P. carinii* aggregates were intensely

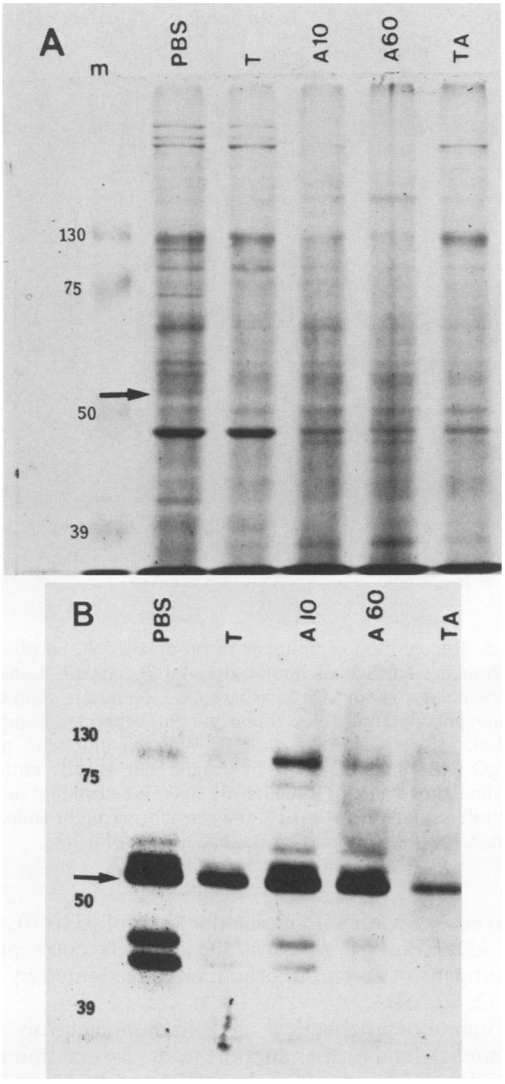

FIG. 2. Precipitates following treatment of *P. carinii* to elute adherent surface immunoglobulins. (A) Coomassie-stained gel. Arrow denotes 52-kDa band visible only in PBS control. (B) Immunoblot with anti-human IgG as probe. Arrow denotes 52-kDa band. Lanes: m; molecular weight markers; PBS, phosphate-buffered saline, pH 7.4; T, Triton X-100 for 10 min; A10, glycine-HCl for 10 min; A60, glycine-HCl for 60 min; TA, Triton X-100 for 10 min followed by glycine-HCl for 10 min.

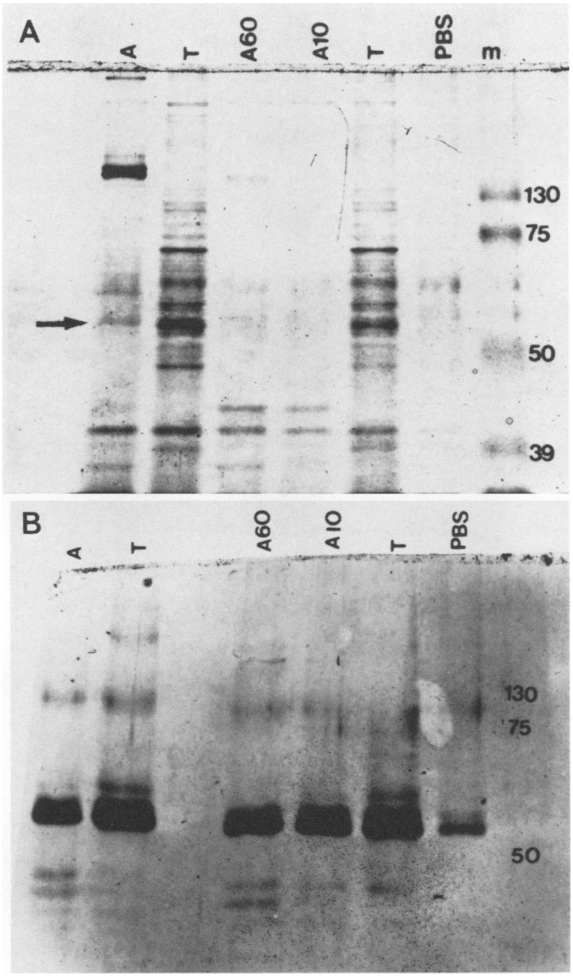

FIG. 3. Supernates following treatment of *P. carinii* to elute adherent surface immunoglobulins. (A) Coomassie-stained gel. Arrow denotes 52-kDa band. (B) Immunoblot with anti-human IgG as probe. Lanes are as described in legend to Fig. 2, except that supernates produced by sequential treatment with Triton X-100 and glycine-HCl were evaluated separately (two left lanes).

positive when reacted with anti-IgG. The A549 cells did not react with anti-IgG, nor was there a colorimetric reaction of the *P. carinii* aggregates with enzyme substrate in the absence of anti-IgG.

At the ultrastructural level, anti-human IgG labeled the electron-dense outer surfaces of cysts as well as fine membranous structures and vesicles adjacent to cysts (Fig. 7). The label was present at discrete sites on the cyst wall. The gold did not label human cells. Exposure to glycine-HCl abolished the binding of anti-human IgG to *P. carinii* cysts.

DISCUSSION

This study demonstrates the presence of immunoglobulins on the surface of *P. carinii* obtained from individuals with AIDS. The methods used were SDS-PAGE and immunoblotting studies, immunocytochemistry, and immunoelectron microscopy. We found that IgG and IgA and only trace amounts of IgM were present. We concentrated on the presence of IgG in our study.

Affinity-purified, anti-human IgG as well as monoclonal antibodies to the Fc and Fab portions of human IgG consistently bound a 52-kDa band present in homogenates of purified, human-derived *P. carinii*. The molecular sizes of human IgG heavy chains are in the 50- to 60-kDa range (20), suggesting that the 52-kDa band may in fact be IgG heavy chains. The 52-kDa band could not be totally eluted. Even after treatment with Triton X-100 in combination with glycine-HCl, a 52-kDa band was visible in the precipitate (Fig. 2). Thus, this band may represent an immunoglobulin fragment that is firmly bound to a surface of *P. carinii*. In contrast, the 45- and 42-kDa fragments that reacted with anti-human IgG were significantly reduced or completely removed by the various elution techniques. These fragments may represent parts of the immunoglobulin G molecule less firmly adherent to the surface of *P. carinii*.

Major immunoreactive bands of 50 kDa (22, 23) and 55 to 60 kDa (9) have been reported by others in rat- and human-

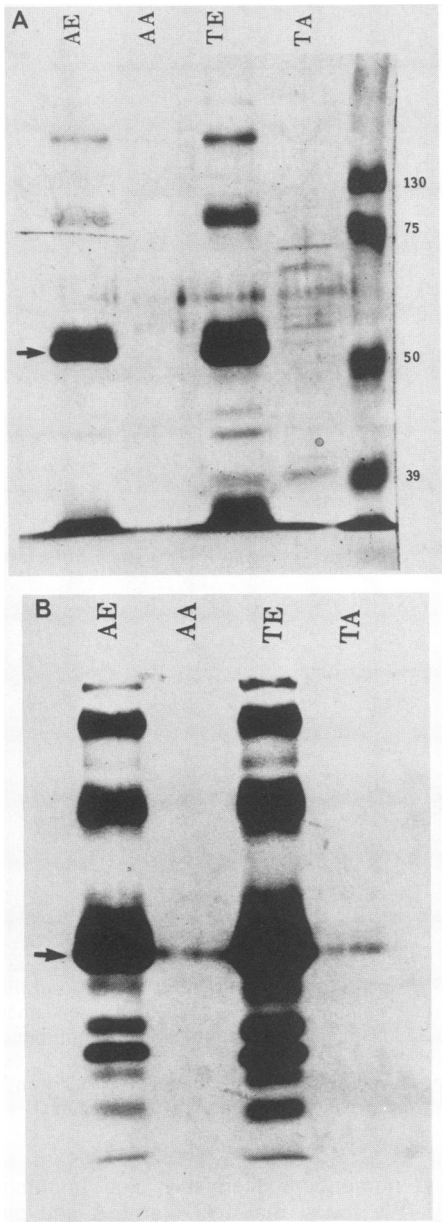

FIG. 4. Purification of IgG from supernates by protein G column chromatography. (A) Coomassie-stained gel; (B) immunoblot with anti-human IgG as probe. The arrow denotes the 52-kDa band. Lanes: TA, nonadherent fraction of supernate produced by Triton X-100; TE, adherent fraction of supernate produced by Triton X-100; AA, nonadherent fraction of supernate produced by glycine-HCl, 60 min; AE, adherent fraction of supernate produced by glycine-HCl, 60 min.

derived *P. carinii*. It is interesting that others have noted the reactivity of conjugated anti-IgG with a 50-kDa band on immunoblots and with whole *P. carinii* organisms even after washing with glycine-HCl (10). Methodological differences relating to the purification of *P. carinii* and gel running and blotting conditions make exact comparison among the studies difficult. However, the multiplicity of bands with approximate molecular sizes of 50 kDa in homogenates of *P. carinii* on Coomassie-stained gels raises the possibility that some of the bands in this molecular size range may be host derived.

The purified IgG in the eluate recognized discrete bands in

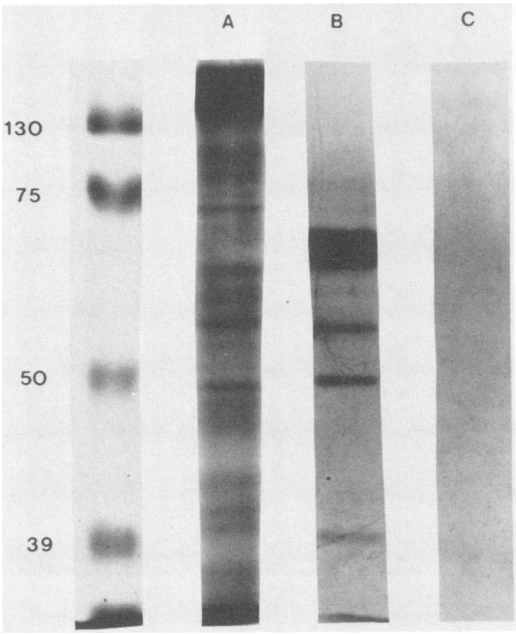

FIG. 5. Recognition of antigens in rat-derived *P. carinii* by IgG eluted from the surface of human-derived *P. carinii*. Lanes: leftmost, molecular size (in kDa) markers; A, Coomassie stain of 7.5% acrylamide gel containing electrophoretically separated components of purified, rat-derived *P. carinii*; B, Western blot with purified, eluted IgG (0.25 mg/ml), 1:500, overnight followed by anti-human IgG-alkaline phosphatase, 1:1000, for 1 h; C, Western blot incubated in Tris-buffered saline with 0.1% Tween 20 overnight followed by anti-human IgG-alkaline phosphatase, 1:1000, for 1 h.

rat-derived *P. carinii* with molecular sizes of 65 to 70, 60, 50, and 35 kDa (Fig. 5). Most of these bands correspond to major antigens of *P. carinii* origin, as documented by others (9, 10, 12, 22, 23).

The immunocytochemical and immunoelectron microscopic studies lend further support to the association of host immunoglobulins with *P. carinii*. Without these additional studies, we could not rule out the possibility that immunoglobulins in the alveolar space or lavage fluid, perhaps in association with host cells, were being purified along with *P. carinii*. The structural studies also show that the immunoglobulins identified by SDS-PAGE and immunoblotting are associated with *P. carinii* and are not merely reflections of immunoglobulin-bearing host cells that may have been present in the preparations of purified *P. carinii*. The immunoelectron microscopy studies show that the external surface of the cyst is focally coated with IgG. Additionally, IgG was present on the fine membranous structures, tubular extensions, and vesicles adjacent to cysts that others have shown to be carbohydrate rich (24). The material that we used for electron microscopy was human bronchoalveolar lavage fluid that had been stored frozen in 20% glycerol in PBS. Cyst morphology was quite good. Although we did see intact trophozoites that were focally labeled by anti-IgG on their external surfaces, we cannot comment on the relative amounts of IgG on the surfaces of cysts and trophozoites because of the possibility that trophozoite morphology was adversely affected by freezing. Although the amount of colloidal-gold-tagged anti-human IgG that bound to *P. carinii* was not plentiful, we did not see any binding whatsoever in the acid-treated preparation. Additional evidence of the tight association between *P. carinii* and host IgG was obtained by

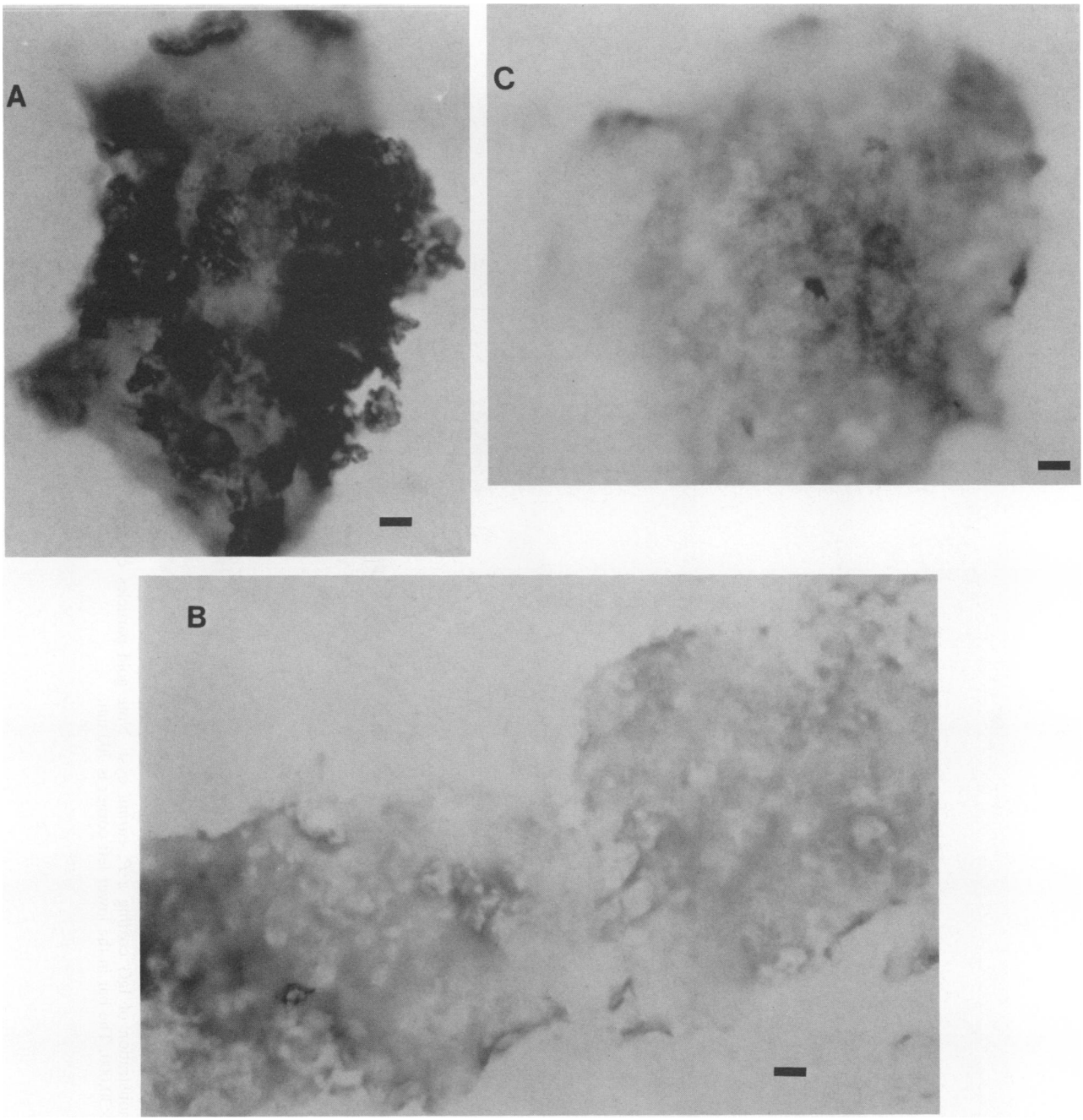

FIG. 6. Immunocytochemical demonstration of IgG coating intact aggregate of *P. carinii* cysts and trophozoites. (A) Anti-human IgG; (B) anti-rat IgG; (C) buffer control. Note strong positivity as visualized by black colorimetric reaction around aggregate in panel A but not in panel B or C. Magnification, ×760. The bar in the lower right corner is 5 μm.

the immunocytochemical study of monolayers to which human-derived *P. carinii* adhered (2). The aggregates of *P. carinii* had been on the monolayers for 3 or 31 h and reacted intensely with anti-IgG, showing that the IgG is associated with *P. carinii* even after 31 h of in vitro cultivation.

Brzosko et al. found surface immunoglobulins, predominantly IgG and IgM, on *P. carinii* obtained from infants with plasma cell pneumonitis in the pre-AIDS era (4, 5). The immunoglobulins bound complement and rheumatoid factor, leading the authors to speculate that there was no defect in host humoral defense mechanisms. Rather, the defect was postulated to be one of complement deficiency, resulting in delayed removal of organisms by macrophages. Merlier et al. studied seven patients with adult *P. carinii* pneumonia, four of whom had underlying hematopoietic malignancies, diagnosed between 1976 and 1981; they documented the presence of surface IgG and IgA by direct immunofluorescence (14). The current study extends the observation to *P. carinii* obtained from AIDS patients. In addition, the current study documents that the eluted IgG, when used as a probe in Western blots (immunoblots), recognizes specific antigens of *P. carinii* origin. Our finding that IgG and IgA are both more plentiful than IgM supports the idea that AIDS-related *P. carinii* pneumonia is either a secondary acquisition or a

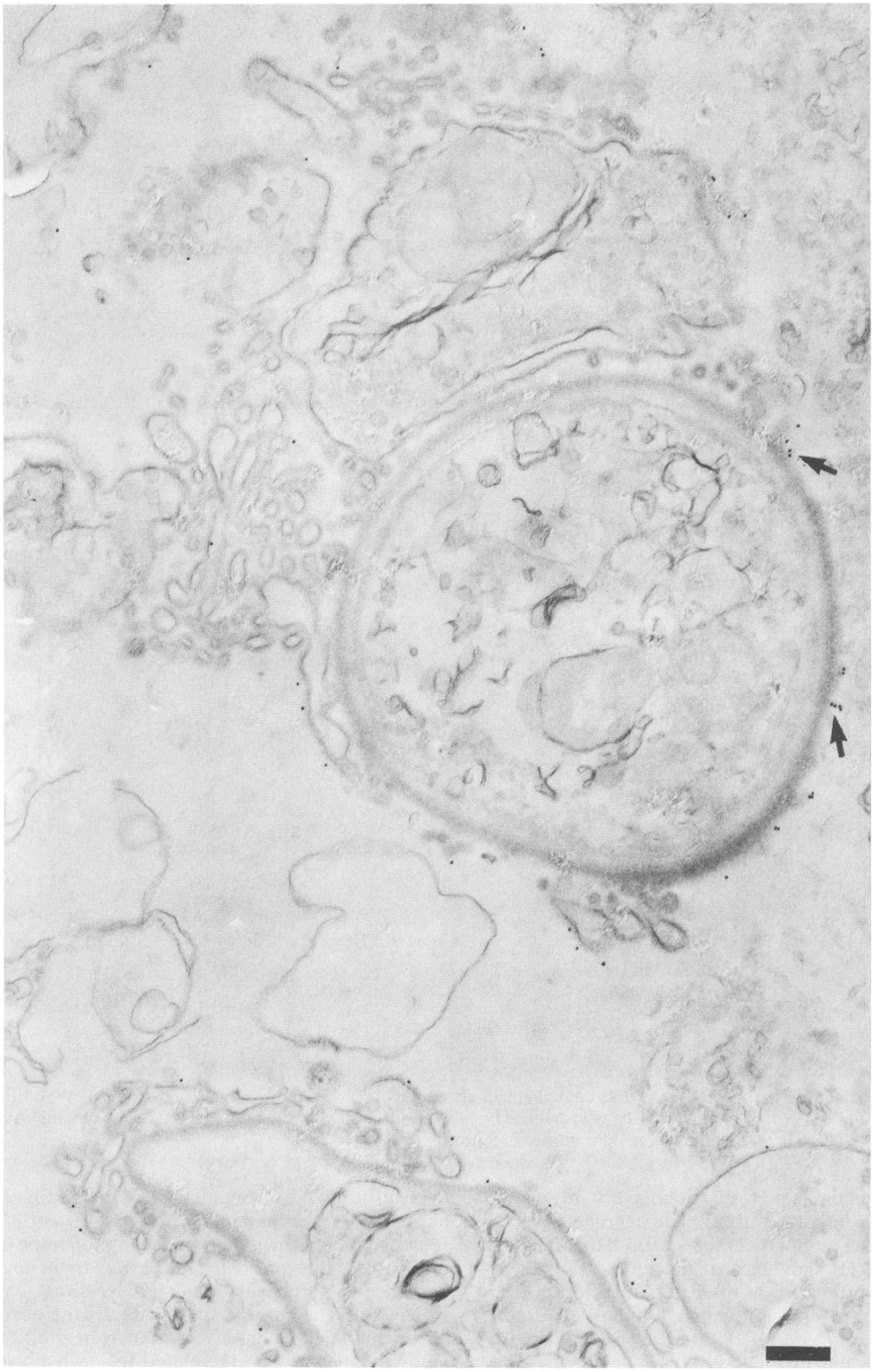

FIG. 7. Immunoelectron microscopic demonstration of IgG coating a *P. carinii* cyst. Note gold particles clustered around the cyst in discrete locations (arrowheads denote representative gold clusters). Magnification, ×30,000. The bar in the lower left corner is 700 nm.

reactivation of latent organisms, unlike the primary infection of plasma cell pneumonitis which is characterized by copious IgG and IgM with trace amounts of IgA. Additionally, plasma cells are rarely prominent in AIDS-associated *P. carinii* pneumonia, suggesting that immunoglobulin production may not be local. Thus, the pathogenesis of *P. carinii* pneumonia in AIDS patients is likely to be quite different from the pathogenesis of *P. carinii* plasma cell pneumonitis in infants.

A number of studies, recently summarized (8), have reported anti-*Pneumocystis* antibodies in the sera of healthy and sick children and adults. AIDS patients have increased levels of serum globulins as well as increased levels of IgG and IgA in bronchoalveolar lavage fluid (19, 25). It is currently unclear how much of this antibody is directed against *P. carinii*. Additional studies may reveal the proportion of the total immunoglobulin present in alveolar fluid that is represented by immunoglobulin on the surface of *P. carinii*.

The role, if any, of surface IgG in the pathogenesis of *P. carinii* pneumonia in AIDS patients is currently not known. If the IgG on the surface of *P. carinii* is an opsonizing antibody, then it would be reasonable to hypothesize that HIV-related immune defects in macrophages or lymphocytes are in some way responsible for the proliferation of *P. carinii* in AIDS patients. On the other hand, the antibody coating the surface of *P. carinii* may function as a blocking antibody and may mask immune detection of surface *P. carinii* antigens. This latter possibility seems less likely but should be entertained, given the current lack of knowledge. Further work is required to characterize the other host components that may be present on the surface of *P. carinii* and to determine the functional role of these components.

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