Immunoscintigraphy of *Pneumocystis Carinii* Pneumonia in AIDS Patients

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The diagnosis of *Pneumocystis carinii* pneumonia (PCP) currently relies upon cytological demonstration of the organism in sputum or bronchoscopy specimens. The purpose of this study was to develop a radiolabeled monoclonal antibody (Mab) against *Pneumocystis carinii* (*P. carinii*) and to evaluate its use for imaging PCP. **Methods:** We studied 16 HIV-infected patients with pneumonia in order to evaluate a new Mab-based imaging method for diagnosing PCP. Most patients were managed for opportunistic pneumonia associated with AIDS, including standard cytological tests, and, in all cases, intensive chemotherapy. Prior to the clinical study, the Mab raised to *P. carinii* was shown to react with human *P. carinii* but not with rat *P. carinii* or human white blood cells. **Results:** After labeling a 1-mg Mab Fab' fragment with 30 mCi of ^{99m}Tc, the presence or absence of PCP could be confirmed in six of seven or seven of eight assessable patients, respectively, by external photoscanning within 24 hr. This shows a sensitivity of 85.7% and a specificity of 86.7%. **Conclusion:** Our findings suggest that PCP can be diagnosed by a noninvasive imaging method employing a small dose of a ^{99m}Tc-labeled Mab showing specificity for the infectious organism, since patients with *P. carinii*-free pneumonia were correctly negative in 87.5% of cases. Rapid diagnosis and organ-localization of other infectious lesions with organism-specific, radiolabeled Mabs may be feasible.

Key Words: antibody imaging; diagnosis; immunoscintigraphy; *Pneumocystis carinii* pneumonia; radioimmunodetection

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Pneumocystis carinii is a ubiquitous human commensal organism affecting immunocompromised individuals, such as those with acquired immunodeficiency disease syndrome (AIDS), transplant recipients, patients receiving cytotoxic chemotherapy, malnourished newborns and infants with hypogammaglobulinemia, thymic dysplasia or severe combined immunodeficiency (*1*). With the advent of the AIDS epidemic, PCP has increased in incidence and in importance, reflecting its place as the major opportunistic infection of the HIV-infected individual (*2*). It has been estimated that about 80% of AIDS patients experience PCP at some point in their illness (*3*). In addition to the introduction of zidovudine and other therapeutic agents enhancing survival of AIDS patients, earlier PCP diagnosis probably plays an important role in the successful management of this opportunistic infection, since earlier diagnosis leads to specific anti-PCP therapy when the patient is less ill and more likely to respond favorably. Increased survival of AIDS patients is likely attributable to the improved management of PCP as well.

The past decade has seen a number of tests and diagnostic modalities developed and examined in an attempt to gain reliable and specific measures beyond chest radiography and arterial oxygen tension to diagnose pneumonia due to *P. carinii* organisms. Early in the AIDS epidemic, bronchoscopy with biopsy or bronchoalveolar lavage (BAL), and occasionally open-lung biopsy, were undertaken to obtain material from which a definitive cytological diagnosis could be made (*4*). These methods, however, were attended by increased patient risk, pain and expense. Hence, there has been a search for noninvasive procedures, including gallium scans and pulmonary function tests. Unfortunately, the latter approaches lack specificity, since other pulmonary inflammatory processes also demonstrate abnormalities (*3–6*). Ultimately, the diagnosis of PCP rests upon demonstration of the organism in pulmonary secretions or tissues. Although bronchoscopy with BAL (which can include transbronchial biopsy) is the standard method for securing samples for cytological analysis, sputum can be induced from AIDS patients, and by means of special stains, about 55% or more of patients can be diagnosed by this noninvasive test (*7,8*). The development of monoclonal antibodies (Mabs) against *P. carinii* has improved the sensitivity and specificity of sputum cytology (*9*). However, much skill is required for effective sputum acquisition, processing and interpretation.

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There is, therefore, an understandable need to develop a rapid, safe, specific, noninvasive test for PCP, which is the subject of this study. Past research in cancer imaging with radiolabeled antibodies made against tumor antigens or "markers" has indicated that tumors can be imaged by the radioimmunopharmaceutical (*10,11*). We report the results of a series of 16 HIV-infected patients evaluated for PCP in whom the value of a radiolabeled anti-*P. carinii* Mab for disclosing *P. carinii* infection by external scanning methods was appraised.

MATERIALS AND METHODS

Human *P. carinii* isolated from the lungs of a patient with AIDS was used to immunize CB6F1/J mice (Jackson Laboratories, Bar Harbor, ME), and when the mice were in the log phase of antibody production (as analyzed by immunoblotting), they were injected intraperitoneally with the antigen preparations for 3 days prior to fusion. Spleen cells from the mice were fused with the nonimmunoglobulin-producing myeloma cell line, P3-X63/Ag8.653 (BALB/c origin), and hybrids selected according to conventional methods. Resulting hybridomas were screened for *P. carinii* reactivity by an immunoblotting procedure described previously (*12*). Hybrids which demonstrated reactivity against *P. carinii* were dilution-cloned twice and injected into mice for production of ascites fluid. Of the six clones determined to be reactive with *P. carinii*, one was an IgG_{1K}, designated 7C5 and was selected for further development.

Surface reactivity of the 7C5 Mab against humanized *P. carinii* was determined by indirect immunofluorescence methods. Reactivity to specific *P. carinii* antigens derived from human and rat isolates was determined by immunoblotting. The Mab 7C5 was screened for crossreactivity by dot-blot and/or immunofluorescence with the following organisms: *Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Candida albicans, Cryptococcus neoformans, Microsporum canis, Trichophyton mentagrophytes, Aspergillus fumigatus, Histoplasma capsulatum, Blastomyces detmatitidis* and *Leishmania sp.*

Flow cytometry (Beckon Dickinson FACScan, Mountainview, CA) was used to assess the reactivity of 7C5 with normal human white blood cells from a healthy donor. After incubation at 4°C for 30 min and washing, the cells were incubated with fluorescein-conjugated goat antimouse IgG and analyzed in gated windows to examine reactivity with granulocytes, lymphocytes and monocytes in comparison to known positive and negative control antibodies. No reactivity was found.

Mab 7C5 was purified from mouse ascites of pristane-primed BALB/c mice (Harlan, Indianapolis, IN) using Protein A (Repligen, Cambridge, MA) and S-Sepharose (Pharmacia, Piscataway, NJ) chromatography. The purity of the 7C5 preparation was confirmed by SDS-PAGE under reducing and nonreducing conditions, size-exclusion high-pressure liquid chromatography (HPLC) (Zorbax GF-250; MacMod, Chadds Ford, PA), and by immunoelectrophoresis. The whole IgG preparation was sterile, pyrogen-free by the Lymulus lysate test (QCL-100; Whittaker, Walkersville, MD), general safety tested according to the Code of Federal Regulations' Chapter 21, Part 610.12, and free of murine viruses by mouse antibody production testing. The whole IgG was subsequently digested with pepsin, and purified by Protein A and ultrafiltration (Amicon YM30; Danvers, MA). The $F(ab')_2$ fragments were reduced to Fab' fragments and then formulated for direct labeling with ^{99m}Tc (*13*). The formulated product was stored in 1.0-mg aliquots (0.75 ml) in the vapor phase of liquid nitrogen. Immediately prior to the addition of ^{99m}Tc-pertechnetate, a vial was thawed at room temperature, and approximately 30–40 mCi of $^{99m}TcO_4$ (Medi-Physics, Livingston, NJ) was added in 1.0–1.2 ml. After 15–30 min, the material was ready for injection. Quality assurance on each ^{99m}Tc-labeled product included HPLC, instant thin-layer chromatography (ITLC) for the detection of unbound ^{99m}Tc and colloid and tests for sterility and pyrogenicity. All labeled products were shown to be sterile and pyrogen-free.

Instead of determining the antibody's immunoreactivity by quantitative tests, the postlabeled reactivity with the *P. carinii* epitope was compared after ^{99m}Tc labeling to that of an ^{125}I-labeled antibody preparation by Western blot analysis, using SDS-PAGE minigel transferred to mitrocellulose strips. Iodine-125-labeling was performed by the chloramine-T method to a specific activity of 10 mCi/mg (*14*). The strips were dried and exposed overnight at −80°C to Kodak Xomat AR film with a single intensifying screen.

HIV-infected patients presenting with clinical signs and symptoms as well as roentgenographical evidence of pneumonia suspected or proven to be PCP were eligible. Patients were recruited as part of their evaluation for pneumonia, and virtually all were already undergoing chemotherapy for PCP, and in some cases other putative infections, prior to or at the time of referral for study. Except for Patients 1353 and 1390, who were on pentamidine, all patients were on trimethoprim-sulfamethoxazole (TMP-SMX) therapy. In Patients 1312 and 1392, pentamidine was also administered. The patients were all HIV-1-seropositive adults, 12 males and four females ages 23–41 years, whose most common risk factor was parenteral illicit drug use. All patients were receiving at least 300 mg daily of zidovudine. Their absolute $CD4^+$ counts were $<115/\mu l$ at entry. Sixteen patients were recruited from December, 1991 through April, 1993. Protocols were approved by the Center for Molecular Medicine and Immunology Institutional Review Board and written informed consent was obtained from all patients. Diagnostic analyses by cytology were performed in all cases (by sputum in 8, BAL in 13 patients, and transbronchial biopsy in 4 patients). When sputum specimens failed to reveal *P. carinii* and BAL or transbronchial biopsy proved positive, the positive result was considered confirmatory of pneumocystosis. Only one patient refused to complete the study (No. 1312) and thus was considered unassessable. Baseline clinical laboratory data, including complete blood cell counts and differentials, serum electrolytes, lactate dehydrogenase (LDH), creatinine, liver function enzymes and T-cell profiles, were obtained.

The patients were given Perchlorocap (200 mg $KClO_4$ U.S.P.) twice daily starting 30 min prior to the infusion of the radioantibody, and ending 1 day later. The patients were infused intravenously after the placement of an Angiocath; 45 ml of saline were transferred to a buretrol solution set chamber, and after ensuring that the apparatus was performing smoothly, 25–30 mCi (about 1.0 mg) ^{99m}Tc-7C5 Fab' were added to the chamber. The initial flow rate was at 0.5 ml per min for 5 min. Vital signs were then obtained, and if no adverse reaction was noted, the remaining volume was infused over 15 min. Imaging was performed on a large field-of-view Anger camera with a GAP collimator (DS-X camera from Sopha Medical Systems, Columbia, MD). One million counts for anterior and posterior planar images were taken of

the head, chest, abdomen and pelvis at 2 hr and at 5 hr. Planar images were repeated at 24 hr but for 20 min per view. Anterior and posterior whole-body images were also obtained with a fixed scan speed of 120 cm/min at each time point to find the total-body radioactivity clearance rate. A 64 × 64 matrix, 128 angles and 10 sec/angle, chest SPECT acquisition was performed at 4 hr postinjection. The SPECT data were processed for transverse, coronal and sagittal slices using a Hamming-Hann backprojection filter. The images were read by two or three nuclear medicine physicians who were blinded to the patients' clinical history and present status. After recording their readings, the data were correlated with other clinical and laboratory information, after which sensitivity, specificity and accuracy values were computed. Activity in the lungs was considered to be increased when it exceeded the activity in adjacent tissues in the chest. The RAID scans were compared with chest radiographs (all patients) or ^{67}Ga scans (Patients 1311, 1353, 1355, and 1371), but final correlations were made on the basis of cytological findings from induced sputum, BAL and/or transbronchial biopsies, except in Patients 1338 and 1369, where the correlation was made on a clinical basis.

RESULTS

Immunofluorescence and immunoblotting results with the Mab 7C5 were in agreement when tested against human and rat *P. carinii* specimens. The antibody did not react with the rat-derived specimen, and reacted with human isolates. Flow cytometry studies with leukocytes from a healthy donor did not show any reactivity with circulating neutrophils, lymphocytes, or monocytes.

By Western blot analysis of human *P. carinii*, radioiodinated 7C5 detected multiple bands of molecular weights between 40,000 and 120,000 (Fig. 1). The most prominent reactivity was with a band of approximately 116,000 which appeared to correspond to an important surface antigen of *P. carinii* which has been termed "major surface glycoprotein (MSG)" or "glycoprotein A (gpA)." The molecular weight of this antigen has varied from 95,000 to 140,000 in different studies, depending on host of origin and method of purification (*15*). It has also been shown that multiple genes encode for this antigen, suggesting the possibility of antigenic variation (*16*). The lower molecular weight bands detected by 7C5 could either represent breakdown products of the 116,000 band or an epitope recognized on multiple proteins. 7C5 did not react with the panel of bacteria, fungi, and protozoa. After labeling with ^{99m}Tc, 7C5 showed identical reactivity by Western blotting to the ^{125}I-labeled preparation, indicating no apparent change in the antigen-binding after direct labeling with ^{99m}Tc (Fig. 1).

After labeling with ^{99m}Tc, 91% of radioactivity was found by HPLC to be bound to the Fab' fragments, with less than 6% of radioactivity associated with residual $F(ab')_2$ and no more than 2.4% representing unbound ^{99m}Tc (results not shown). ITLC results indicated an average of 4.8% ± 3.3% unbound ^{99m}Tc and less than 2% colloid. Fab' fragments of 7C5 were prepared and formulated for direct labeling with ^{99m}Tc according to methods described previously (*13*). Size-exclusion HPLC profiles using both optical density and in-line radiation detectors showed almost complete incorporation of the ^{99m}Tc in the 7C5 Fab'.

Western blot showing the reactivity of radiolabeled 7C5 against SDS-solubilized human *P. carinii*.

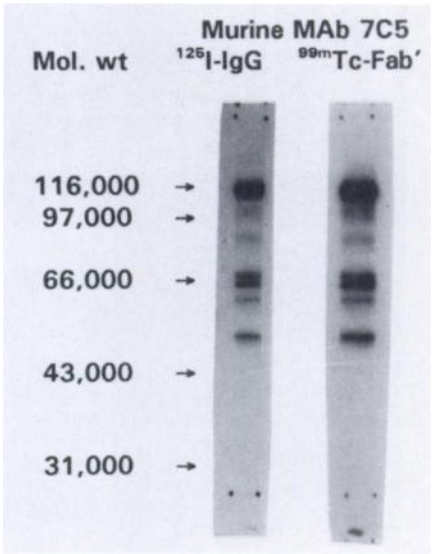

FIGURE 1. Western blot analysis of ^{125}I-labeled 7C5 IgG with SDS-solubilized human *P. carinii*. Reactivity with multiple bands is evident. The range of molecular weights was based on standards run on a parallel lane.

The antibody imaging results are provided in Table 1 with their correlation to cytological evidence of the presence of *P. carinii* in the lungs. Of the fifteen assessable patients, six proved to be true-positive by antibody imaging, seven were true-negative, one was false-positive (no. 1311) and one was false-negative (no. 1354). This provides a sensitivity (true-positive/true-positive + false-negative) of 85.7%, a specificity (true-negative/true-negative + false-positive) of 87.5% and an accuracy (true-positive + true-negative/total) of 86.7%. Examples of true-negative and true-positive imaging results are shown in Figures 2 and 3, respectively. Although antibody imaging studies were performed at several times, particularly at 2–5 hr and 24 hr after injection, it was found that the 24-hr planar imaging studies generally provided the most reliable results, despite the availability of both planar and SPECT scanning findings at the earlier imaging times. Diffuse activity in the lung was observed in four patients (nos. 1307, 1355, 1369 and 1392), a diffuse heterogenous uptake bilaterally in the lower lung fields of one patient (no. 1311), focal uptake in the left lower lung and diffuse uptake in the right lung (Patient 1356), and both focal and diffuse uptake in the apices of both lungs and the base of the left lung of another patient (no. 1401). No extrapulmonary sites of abnormal radioactivity were observed in any of the patients. No laboratory findings or clinical signs of toxicity or adverse reactions related to the antibody administration were noted in any of the patients.

TABLE 1
RAID Results for PCP in AIDS Patients

Patient no.	Sex	Age (yr)	CD4 titer cells/μl	pO_2 mmHg	LDH (U)	Cytology results			RAID			Correlation	Outcome and notes
						Sputum	BAL	TBB	Results	Days post-Dx	Days post-Rx		
1307	M	42	13	73	193	ND	POS	ND	POS	4	11	TP	Rx: successful
1311	M	41	0	—	>1000	ND	NEG	ND	POS	9	16	FP	^{67}Ga-POS; TB: POS in gastric aspirate; Candida: POS by BAL; Expired
1312	M	35	—	41	765	NEG	POS	ND	NA	3	13	NA	Incomplete RAID study
1314	M	27	8	64	217	ND	NEG	NEG	NEG	5 days pre-Dx	6	TN	Dx: Organized pneumonia
1338	M	28	8	62	593	ND	POS	POS	NEG	15	28	TN*	Rx: successful; N.E.D. post-Rx and at RAID
1353	F	33	9	93	123	NEG	NEG	ND	NEG	15	17	TN	^{67}Ga-POS
1354	F	23	7	61	218	NEG	POS	POS	NEG	8	17	FN	Dx: clinical improvement
1355	M	32	115	53	313	NEG	POS	ND	POS	1	13	TP	^{67}Ga-POS; Rx: successful
1356	M	29	—	49	247	NEG	POS	ND	POS	19	28	TP	
1359	M	38	8	59	230	ND	NEG	ND	NEG	7	14	TN	
1369	M	41	48	81	269	ND	IE	ND	POS	13	32	TP*	
1371	M	30	38	—	198	NEG	ND	ND	NEG	13	16	TN	^{67}Ga-POS; CMV seropositive
1390	M	37	15	74	390	NEG	NEG	NEG	NEG	7 days pre-Dx	26	TN	HIV nephropathy → acute renal failure; Expired
1392	F	26	—	69	412	ND	POS	ND	POS	7	20	TP	
1401	F	38	14	60	287	ND	POS	ND	POS	27	29	TP	Rx: successful
1425	M	36	—	67	262	NEG	ND	ND	NEG	14	21	TN	

BAL = bronchoalveolar lavage; TBB = transbronchial biopsy; RAID = radioimmunodetection; ND = not done; NA = not available; IE = inevaluable; TP = true-positive; FP = false-positive; TN = true-negative; FN = false-negative; Dx = diagnosis; Rx = therapy; N.E.D. = no evidence of disease.

*Correlation made clinically.

Among the seven patients who had true-negative results, evidence of agents or processes other than *P. carinii* could account for their clinical symptoms and signs. Therefore, it appears that the imaging Mab is quite organism-specific. For example, Patient 1314's clinical presentation was typical of pneumocystosis. Yet, BAL failed to reveal *P. carinii* organisms and the RAID study was negative 4 days before BAL and transbronchial biopsy. Transbronchial biopsy found only an organized pneumonia, suggesting successful antimicrobial treatment of an unidentified bacterial infection. The one false-positive case (Patient 1311) in fact posed a difficult and uncertain clinical interpretation. This AIDS patient presented with bilateral interstitial pulmonary infiltrates and a $CD4^+$ titer of 0. Treatment with TMP-SMX and erythromycin was started immediately. Fluconazole was added later to the regimen. One week after chemotherapy, BAL was performed, revealing numerous alveolar macrophages, inflammatory cells and eosinophilic conglomerates suggestive of *P. carinii* infection. However, the Grocott methenamine silver stain was negative for *P. carinii* while revealing Candida. Two weeks thereafter, a RAID study showed diffuse heterogeneous radioactivity bilaterally, which was interpreted as a positive scan. The patient died 2 wk later, and several weeks following his death, culture evidence of *Mycobacterium tuberculosis* from a gastric aspirate taken at autopsy was reported. Since the BAL did not reveal *P. carinii* organisms, the correlation was reported as false-positive, although the clinical impression was that of a patient who harbored several organisms, including cytologically undetected *P. carinii*, which did not respond to chemotherapy. The false-negative patient (Patient 1354) presented a similarly complex scenario. This patient also was profoundly $CD4^+$ lymphopenic (7 cells/μl) and was hospitalized with fever, shortness of breath, hypoxemia (pO_2, 61 mm Hg), and a chest x-ray showing bilateral interstitial infiltrates. TMP-SMX was initiated immediately, as was fluconazole. One week later, induced sputum cytology failed to show *P. carinii* organisms, but a BAL 3 days later was Grocott stain positive for *P. carinii*. Transbronchial biopsy at the

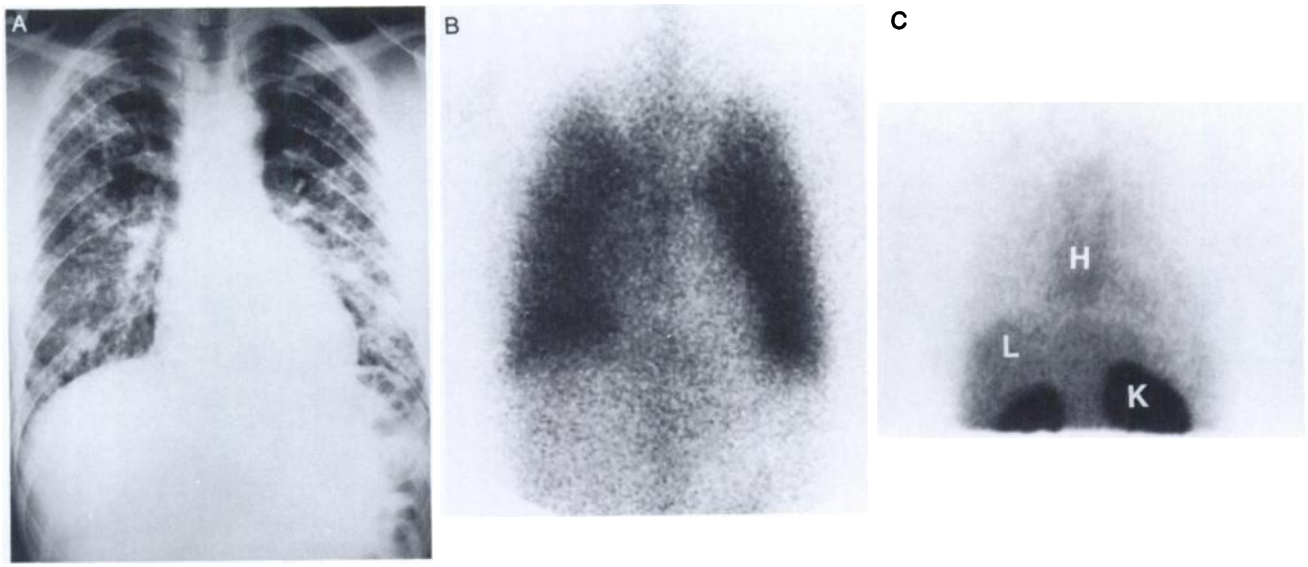

FIGURE 2. Diagnostic studies of an HIV-infected patient (#1371) presenting with suspected PCP that proved to be cytologically negative for *P. carinii*. (A) PA chest roentgenogram shows diffuse bilateral pulmonary infiltrates; (B) 48-hr ^{67}Ga scan shows diffuse bilateral pulmonary uptake; (C) 5-hr anterior chest antibody scan shows no pulmonary uptake. H = heart; L = liver; K = kidneys.

same time also confirmed *P. carinii*. One week later, the RAID study was read as negative. The patient is alive as of the last observation, 8 mo after discharge from this admission. Thus, although this was recorded as a false-negative case, it is conceivable that the patient was successfully treated for PCP and retained no detectable organisms by the time the antibody imaging study was performed.

Two of the correlations were made solely on the basis of clinical data and interpretation. Patient 1338 was admitted with right upper lobe lung infiltrates on chest roentgenography and was immediately given a course of cefuroxime, ampicillin, ceftriaxone and TMP-SMX. He underwent a BAL procedure 9 days later, which revealed a positive Grocott methenamine silver staining for *P. carinii*. Twenty-four days following the onset of chemotherapy and four days before a negative RAID study was obtained, the patient's chest radiograph showed significant improvement from that obtained at admission. Therefore, it was concluded that the negative RAID study was consistent with the clinical outcome following intensive chemotherapy despite a positive cytological finding for *P. carinii* 15 days earlier. In the second instance, Patient 1369 presented with bilateral interstitial infiltrates of a reticular nodular pattern consistent with pneumocystosis on chest roentgenography, and despite empiric therapy for tuberculosis, community-acquired pneumonia, sepsis and *P. carinii* (rifampin, pyrazinamide, ethambutol, isoniazid, ampicillin, gentamycin, vancomycin and TMP-SMX), the antibody imaging study showed positive diffuse uptake of radioactivity bilaterally. Unfortunately, the special staining of the BAL spec-

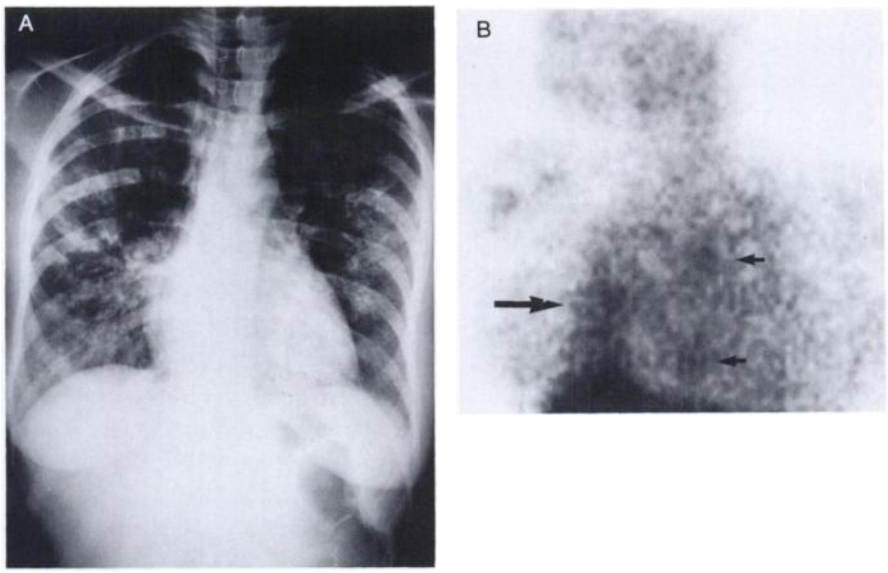

FIGURE 3. Diagnostic studies of an HIV-infected patient (no. 1392) presenting with confirmed PCP. (A) PA chest roentgenogram showing bilateral diffuse patchy interstitial and alveolar infiltrates. (B) Twenty-four hour anterior chest antibody scan shows diffuse uptake in right lower lobe with possible extension into the middle lobe (large arrow) and two areas of focal uptake in the left lung (small arrows).

imen was inadequate, requiring that the true-positive correlation be made on the basis of the clinical presentation.

It is apparent that a particular problem encountered in this study was the variability in the time RAID studies performed after cytological diagnosis or onset of therapy (Table 1). The nature of the patient population investigated presented difficulties in standardizing a protocol. Nevertheless, except for the few exceptions noted, an excellent correlation of RAID with cytological evidence of the presence or absence of *P. carinii* was found.

DISCUSSION

PCP remains the most common presenting feature of AIDS, and continues to account for significant morbidity and mortality despite the availability of effective therapy and prophylaxis. Indeed, it has been estimated that there would be 160,000 cases of PCP in the U.S. by 1991 (*17*). Unfortunately, PCP has no unique hallmark to distinguish it from pneumonia due to a panoply of other agents as well as noninfectious disorders, including but not limited to infections by other viruses (cytomegalovirus, herpes simplex virus), fungi (*Candida, Cryptococcus*), mycobacteria and other bacterial pathogens, protozoa (e.g., *Toxoplasma gondii*), etc. Definitive diagnosis rests on cytological identification of the *P. carinii* organism in sputum or broncheoalveolar specimens by use of special stains designed to detect the trophozoite, intracyst sporozoite forms or the cyst wall (*18*). Induced sputum tests, while more convenient and less costly than BAL, still require a period of 2 to 4 hr until ready for interpretation, are labor-intensive, are not all successful in yielding appropriate specimens for analysis, and have shown adverse reactions, especially in debilitated patients (*19,20*).

The use of Mabs for the detection of organisms has increased the accuracy of cytological identification of *P. carinii* in sputum specimens (*9*). Unfortunately, the accuracy of diagnosis from induced sputum samples, similar to BAL specimens, varies by institution, patient population and the interests, efforts and abilities of the personnel. For BAL to be optimal, the lavage fluid should be obtained from the area of the lung that shows the most extensive disease, not from random sites. When properly performed, BAL material will reveal *P. carinii* in more than 90% of patients with PCP (*21*). Recently, genetic probes for detecting *P. carinii* have been described, but are still somewhat complicated to perform (*22–25*).

The use of radiolabeled Mabs for scintigraphic imaging of cancers with antibodies against tumor-associated markers, of infectious and inflammatory lesions with anti-granulocyte antibodies, of myocardial infarcts and degeneration with anti-myosin antibodies, and of thrombi with anti-fibrin or anti-platelet antibodies (*26*), stimulated us to ask whether this noninvasive diagnostic approach can be applied to the detection of specific pathogens using organism-specific, radiolabeled antibodies. In this study, we have described the development of a murine Mab showing specificity for *P. carinii* organisms derived from human specimens and no cross-reaction with other infectious agents, including bacteria and fungi, as well as human leukocytes. After labeling the reduced Fab′ fragment of the Mab with ^{99m}Tc, which is the preferred radionuclide for nuclear medicine imaging (because of availability, low price and excellent photon energy for scanning), no change in the molecular size or antigen binding of the radiopharmaceutical was found (data not shown). Intravenous injection of a small protein dose of about 1 mg of the Fab′, conjugated with 30 mCi of ^{99m}Tc, into 15 assessable HIV-positive, immunocompromised patients with pneumonia demonstrated PCP with a sensitivity of 85.7% (true-positive in six of seven) and a specificity of 87.5% (true-negative in seven of eight patients). Image interpretation appeared to be optimal with planar scans at 24 hr, thereby not requiring more elaborate and time-consuming emission tomography at earlier scanning times. Thus, it appears from this initial series of patients presenting with suspected PCP that there was a high sensitivity and specificity for diagnosing PCP by external scintigraphy within 24 hr of injecting an antibody radiolabeled to *P. carinii*. In contrast, other imaging modalities, such as roentgenograms and gallium scans, cannot differentiate *P. carinii* from other pulmonary infiltrates, and in the case of gallium, imaging is usually delayed for up to about 3 days with a low specificity achieved (*6,7*). In studies of PCP and other infections in animals and humans, irrelevant polyclonal IgG labeled with ^{111}In showed positive imaging, suggesting a nonspecific uptake in infection (*27,28*). This does not appear to occur in our series of patients with non-PCP pneumonia given our *P. carinii* Mab fragment labeled with ^{99m}Tc. Nevertheless, it is our plan to study PCP patients with a nonspecific Fab′ reagent.

P. carinii may persist in the lungs after apparently successful therapy, which may confuse the evaluation of response (*29,30*). In this situation, a noninvasive test, if it could determine the presence of viable *P. carinii* organisms or their recurrence, would be helpful as a monitor of therapy. Such repeated studies with this murine Mab fragment may be feasible without invoking human anti-mouse antibodies (HAMA), because HAMA reactions have been virtually absent in cancer and infectious disease patients given similar Fab′ preparations (*31*).

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REFERENCES

1. Walzer PD. Pneumocystis carinii—new clinical spectrum? *N Engl J Med* 1991;324:263–265.
2. Martinez A, Suffredini AF, Masur H. Pneumocystis carinii disease in HIV-infected persons. In: Wormser GP, ed. *AIDS and other manifestations of HIV infection*. 2nd ed. New York: Raven Press, Ltd.; 1992:225–247.
3. Murray JF, Garay SM, Hopewell *P. carinii*, Mills J, Snider GL, Stover DE. Pulmonary complications of the acquired immunodeficiency syndrome: an update. Report of the Second National Heart, Lung and Blood Institute Workshop. *Am Rev Respir Dis* 1987;135:504–509.
4. Kovacs JA. Advances in the diagnosis of pneumocystis carinii pneumonia. In: Volberding P, Jacobson MA, eds. *AIDS clinical review*. New York: Marcel Dekker, Inc.; 1990:129–147.
5. Coleman DL, Hattner RS, Luce JM, Dodek PM, Golden JA, Murray JF. Correlation between gallium lung scans and fiberoptic bronchoscopy in patients with suspected pneumocystis carinii pneumonia and the acquired immune deficiency syndrome. *Am Rev Respir Dis* 1984;130:1166–1169.
6. Stover DE, Greeno RA, Gagliardi AJ. The use of a simple exercise test for the diagnosis of pneumocystis carinii pneumonia in patients with AIDS. *Am Rev Respir Dis* 1989;139:1343–1346.
7. Pitchenik AE, Ganjei P, Torres A, Evans DA, Rubin E, Baier H. Sputum examination for the diagnosis of pneumocystis carinii pneumonia in the acquired immunodeficiency syndrome. *Am Rev Respir Dis* 1986;133:226–229.
8. Bigby TD, Margolskee D, Curtis JL, et al. The usefulness of induced sputum in the diagnosis of pneumocystis carinii pneumonia in patients with acquired immunodeficiency syndrome. *Am Rev Respir Dis* 1986;133:515–518.
9. Kovacs JA, Ng VL, Masur H, et al. Diagnosis of pneumocystis carinii pneumonia: improved detection in sputum with use of monoclonal antibodies. *N Engl J Med* 1988;318:589–593.
10. Goldenberg DM, Deland FH, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med* 1978;298:1384–1386.
11. Goldenberg DM, Sharkey RM. Radiolabeled antibodies as in vivo cancer probes [Review]. *Int J Oncol* 1993;3:5–11.
12. Walzer PD, Linke MJ. A comparison of the antigenic characteristics of rat and human pneumocystis carinii by immunoblotting. *J Immunol* 1987;138:2257–2265.
13. Hansen HJ, Jones AL, Sharkey RM, et al. Preclinical evaluation of an "instant" ^{99m}Tc-labeling kit for antibody imaging. *Cancer Res* 1990;50:794–798.
14. Weadock KS, Sharkey RM, Varga DC, Goldenberg DM. Evaluation of a remote radioiodination system for radioimmunotherapy. *J Nucl Med* 1989;30:508–511.
15. Gigliotti F. Host species specific antigenic variation of mannosylated surface glycoprotein of pneumocystis carinii. *J Infect Dis* 1992;165:329–336.
16. Kovacs JA, Powell F, Edman JC, et al. Multiple genes encode the major surface glycoprotein of pneumocystis carinii. *J Biol Chem* 1993;268:6034–6040.
17. Kirsch CM, Azzi RL, Yenokida GG, Jensen WA. Analysis of induced sputum in the diagnosis of pneumocystis carinii pneumonia. *Am J Med Sci* 1990;299:386–391.
18. Smith JW, Bartlett MS. Laboratory diagnosis of pneumocystosis. *Clin Lab Med* 1991;11:957–975.
19. Miller RF, Kocjan G, Buckland J, Holton J, Malin A, Semple SJG. Sputum induction for the diagnosis of pulmonary disease in HIV positive patients. *J Infect* 1991;23:5–15.
20. Nelson M, Bower M, Smith D, Bazzard BG. Life-threatening complication of sputum induction. *Lancet* 1990;335:112–113.
21. Weldon-Linne CM, Rhone DP, Bourassa R. Bronchoscopy specimens in adults with AIDS. Comparative yields of cytology, histology and culture for diagnosis of infectious agents. *Chest* 1990;98:24–28.
22. Wakefield AE, Guiver L, Miller RF, Hopkin JM. DNA amplification on induced sputum samples for diagnosis of pneumocystis carinii pneumonia. *Lancet* 1991;337:1378–1379.
23. Lipschik GY, Gill VJ, Lundgren JS, et al. Improved diagnosis of pneumocystis carinii infection by polymerase chain reaction on induced sputum and blood. *Lancet* 1992;340:203–206.
24. Borensztein L, Hatin I, Simonpoli AM, Ugarte E, Girard PM, Jaureguiberry G. An alternative to DNA extraction for the diagnosis of pneumocystis carinii pneumonia by polymerase chain reaction using a new oligonucleotide probe. *Molec Cell Probes* 1992;6:361–365.
25. Olsson M, Elvin K, Löfdahl S, Linder E. Detection of pneumocystis carinii DNA in sputum and bronchoalveolar lavage samples by polymerase chain reaction. *J Clin Microbiol* 1993;31:221–226.
26. Goldenberg DM, Larson SM. Radioimmunodetection in cancer identification. *J Nucl Med* 1992;33:803–814.
27. Fishman JA, Strauss HW, Fischman AJ, et al. Imaging of pneumocystis carinii pneumonia with ^{111}In-labeled non-specific polyclonal IgG: an experimental study in rats. *Nucl Med Commun* 1991;12:175–187.
28. Buscombe JR, Oyen WJG, Grant A, et al. Indium-111-labeled polyclonal human immunoglobulin: identifying focal infection in patients positive for human immunodeficiency virus. *J Nucl Med* 1993;34:1621–1625.
29. Marchevsky A, Rosen MJ, Chrystal G, Kleinerman J. Pulmonary complications of the acquired immunodeficiency syndrome: a clinicopathologic study of 70 cases. *Hum Pathol* 1985;16:659–670.
30. Hopewell PC. Pneumocystis carinii pneumonia: diagnosis. *J Infect Dis* 1988;157:1115–1119.
31. Pinsky CM, Hansen HJ, Sharkey RM, Jones AL, Goldenberg DM. Lack of human antimouse antibody (HAMA) response in patients imaged with ImmuRAID-CEA [Tc-99m] and ImmuRAID-MN3 [Tc-99m] [Abstract]. *J Nucl Med* 1993;34:214P.

EDITORIAL

PCP, AIDS and Nuclear Medicine

Two articles in this issue of the *Journal* deal with one of the most common complications of AIDS: *Pneumocystis carinii* pneumonia or PCP. In one, Katial et al. (*1*) describe a somewhat less common presentation of PCP on a ^{67}Ga scan. In the other, Goldenberg et al. (*2*) present the first clinical experience in AIDS patients using a radiolabeled antibody specific for PCP. PCP frequently heralds the onset of AIDS in HIV-infected individuals. When AIDS was first recognized, PCP was the presenting opportunistic infection in 60% of cases (*3*) and occurred in over 80% of AIDS patients during the course of their illness. PCP has been and continues to be a significant source of morbidity and mortality in AIDS. It is the first diagnosis considered in a dyspneic or febrile AIDS patient and treatment is often begun empirically.

In the untreated patient, the typical clinical presentation of PCP in the AIDS patient may be quite subtle with little more than a dry cough; fever or dyspnea occur commonly as well (*4,5*). The chest radiograph may be negative (*6*) or may show bilateral and diffuse interstitial markings, alveolar consolidation or "ground glass" opacities (*7,8*). Atypical unilateral infiltrates and cystic changes have been described also (*8*). These cystic changes have been related to an increased incidence of pneumothorax in these patients (*9,10*). Scintigraphic techniques, in general, are extremely sensitive for detection of PCP (*6,11–*

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