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Pneumocystis carinii Antigenemia in Adults with Malignancy, Infection, or Pulmonary Disease

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A counterimmunoelectrophoresis test for *Pneumocystis carinii* antigenemia was employed to assess the extent of subclinical infection or colonization with this agent in adults with infection, pulmonary disease, or malignancy and in healthy homosexual men. Antigenemia was detected in 6 of 208 (3%) of normal controls, 3 of 28 (11%) of patients with pulmonary infection, 3 of 33 (9%) of those with chronic lung disease, 1 of 36 (3%) of patients with lung cancer, 7 of 271 (3%) of afebrile subjects with malignancy, 6 of 19 (32%) of febrile patients with malignancy, 2 of 22 (9%) of those with nonpulmonary infection, and 0 of 21 (0%) of healthy young homosexual men. These data suggest that *P. carinii* is a common commensal or saprophyte that becomes clinically significant only when host defenses are impaired. Antigenemia may occur intermittently during various disease states in the absence of positive clinical signs and should alert the physician to subacute infection or colonization. Treatment appears advisable when clinical data and counterimmunoelectrophoresis results concur.

The need for a dependable noninvasive method for detecting *Pneumocystis carinii* infection has grown with increasing awareness of the ubiquity of this opportunistic agent. Although it was once believed to be rare and a threat primarily to debilitated infants and acutely immunocompromised patients with malignancy, two independent serological surveys have shown that 80 to 90% of normal children have specific antibody to this organism by 2 to 4 years of age (13, 18). Numerous postmortem studies of randomly selected adults have shown that the incidence of asymptomatic infection ranges from 0.2 to 8% (20). The incidence in randomly selected pediatric patients ranges from 0.9% in infants and stillborns to 1% in children under 7 years of age. Adult leukemia-lymphoma patients exhibited a postmortem incidence of *P. carinii* infection of 4.7% (6). The incidence of *P. carinii* pneumonia (PCP) in children in cancer referral hospitals was 13.3 and 5.6%, respectively (15, 21). Recent studies have shown that *P. carinii* was responsible for 18% of mixed or single-agent pneumonias in normal infants less than 3 years of age (22, 23).

The recent increase in the incidence of frequently fatal *Pneumocystis* infections among acquired-immunodeficiency syndrome patients, some of whom exhibit Kaposi's sarcoma (4), intravenous drug abusers (3) and intimate contacts of such individuals (11), hemophiliacs (12), Haitians (1), and infants (2) has emphasized the fact that this agent is considerably more common than previously supposed.

In 1978, Pifer et al. described a counterimmunoelectrophoresis (CIE) test that detects soluble *P. carinii* antigen(s) in the peripheral circulation (18). The test was 95% positive in histologically documented cases, yielded no positive results in 100 normal children, and suggested the presence of subclinical *P. carinii* infection in 15% of 100 randomly selected pediatric patients with cancer.

The ultimate purpose of the present study was to assess the incidence of latent *P. carinii* infections in adult patients with and without malignancy, infection, or chronic lung disease based upon the presence of antigen in the peripheral circulation.

MATERIALS AND METHODS

This study included patients being treated at the University of Tennessee Center for the Health Sciences Hospital and allied clinics and the Veterans Administration Medical Center in Memphis. Patients tested for antigenemia belonged to the following categories: group 1, healthy afebrile adult volunteers; group 2, febrile patients with pulmonary infections; group 3, febrile patients with nonpulmonary infections; group 4, afebrile patients with chronic lung disease; group 5, afebrile patients with lung cancer; group 6, afebrile cancer patients (other than primary pulmonary malignancies); group 7, febrile patients with malignant processes other than lung cancer; and group 8, young afebrile homosexual men. Approximately 5 ml of blood was collected from each patient and control upon admission to the study. Informed consent was signed by all participants. All specimens were coded before being sent to the laboratory for analysis, and serum alone was tested for the presence of antigen.

Clinical data gathered on these patients included history, temperature on admission, hematological profile (leukocytes, hematocrit, differential, and platelets), urinalysis, albumin, globulin, serum protein electrophoresis, blood urea nitrogen, sodium, calcium, potassium, HCO_3, liver function studies, blood gases, blood, sputum and spinal fluid cultures, chest films, any special X-rays or other studies, and drug regimens.

Coded blood specimens were sent to the laboratory where the sera were electrophoresed with antisera raised in rabbits against cell culture-grown *P. carinii* organisms as described in detail previously (1, 17). CIE plates were prepared from 1% SeaKem agarose (FMC Corp., Marine Colloids Div., Rockland, Maine), and electrophoresis was carried out at 50 V and 10 MA for 40 min in barbital buffer (pH 8.2 ± 0.2). Specimen wells, 3 mm in diameter, were filled with 10 μl of antibody or serum. Serum specimens were absorbed with

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Lyphogel (Gelman Sciences, Inc., Ann Arbor, Mich.) for 10 min for the purpose of concentrating antigen that might be present. Approximately five Lyphogel crystals were added for each 0.3 ml of serum absorbed. The appearance of a discrete precipitin band between antigen and antibody wells signified the presence of antigen in the serum of the patients. Positive control serum samples from patients with tissue-documented *P. carinii* were included in every test.

RESULTS

Afebrile healthy adults ($n = 208$) were tested for *P. carinii* antigenemia. Six control subjects or 3% of the total were positive for antigen. Table 1 lists the pulmonary pathology status of these patients and the number with *Pneumocystis* antigenemia. The incidence of *Pneumocystis* antigenemia in patients with pulmonary diseases was from 3 to 11%. The highest incidence of antigenemia in this category was found in febrile patients with primary pulmonary infections (bacterial, fungal, or viral).

Table 1 also lists patients with hematological malignancies or solid tumors (febrile or afebrile) and whether they tested positive for antigen. Patients with malignant processes who were also febrile had a 10-fold higher incidence of antigenemia when compared with normal subjects or afebrile cancer patients.

None of the patients with antigenemia were known to subsequently develop acute PCP, although some expired from other causes or were lost to follow-up. Several exhibited sporadic, intermittent antigenemia. Without exception, all antigen-positive patients, excluding the normal adults in group 1, exhibited one or more of the known risk factors for *Pneumocystis* infection, i.e., immunocompromise secondary to malignancy or therapeutic immunosuppression, or both, chronic lung disease, hyperthermia associated with infection, chronic underlying disease, advanced age, chronic alcoholism, weight loss of more than 10 lb (4.5 kg), or general debility. Of the 21 homosexual men evaluated in the study, none (0%) tested positive for *Pneumocystis* antigen.

DISCUSSION

During the past 8 years few concepts have changed so radically as those concerning *P. carinii*. Originally thought to be a rare pulmonary opportunist manifesting itself solely in acute life-threatening episodes, it is now understood that these cases represent only the tip of the iceberg (13). The prominence of PCP among patients with the acquired immune deficiency syndrome supports this concept (5). This ubiquitous agent is apparently capable of manifesting a spectrum of infections in individuals whose immunological defenses are compromised. The increasing incidence of *Pneumocystis* infection in patients with malignancy emphasizes the need for better epidemiological data concerning this agent and a noninvasive means for detecting and monitoring *P. carinii* infections.

TABLE 1. *P. carinii* antigenemia in various groups of patients and in controls

Description of groups	No. of patients	No. (%) positive for antigen
Pulmonary pathology		
Pulmonary infection (febrile), group 2	28	3 (11%)
Chronic lung disease (COPD,[a] asthma, etc.), group 4	33	3 (9%)
Lung cancer, group 5	36	1 (3%)
Temp status		
Malignancy (afebrile without signs of infection), group 6	271	7 (3%)
Malignancy (febrile), group 7	19	6 (32%)
Nonpulmonary infection (febrile), group 3	22	2 (9%)
Controls		
Normal adults, group 1	208	6 (3%)
Healthy young homosexual males, group 8	21	0 (0%)

[a] COPD, Chronic obstructive pulmonary disease.

The results of previous studies indicated that on the basis of antigenemia data, subclinical infections with *P. carinii* are considerably more common than previously recognized (13, 18). It was further shown that afebrile ambulatory adult cancer patients and those with lung cancer without hyperthermia were no more likely than ostensibly healthy adults to exhibit antigenemia (16). Approximately one-third of the patients with malignancy hospitalized for febrile episodes were found to be antigenemic (19). Nine percent of the individuals without malignancy but with febrile nonpulmonary infections, 9% of those with pulmonary disease (chronic obstructive pulmonary disease, asthma, etc.), and 11% of the febrile patients with pulmonary infections had relative incidences of antigenemia that did not exceed that observed in normal individuals by more than 8% (16).

It is important to attempt to answer in such a survey whether false-positive CIE results occurred in the study. Since biopsy was unfeasible in this survey, as is frequently the case in clinical practice, a definitive answer is not possible. Interpretation of the data must consider such factors as (i) specificity of the antiserum employed in the test, (ii) CIE data derived from biopsy-documented cases of *Pneumocystis* infection, (iii) serological data, (iv) epidemiological data, and (v) clinical data.

Antigen-antibody specificity with regard to *P. carinii* has been well documented (17, 18). The Centers for Disease Control recently verified the specificity of the CIE test for *P. carinii* antigenemia and corroborated our conclusion that subclinical infections with this agent are more common than previously believed (9, 10). The Centers for Disease Control group has not, however, achieved with the CIE test a level of sensitivity comparable to that obtained by our methods (9, 10). In parallel tests performed in our laboratory at the request of Maddison et al. (10), it was apparent that the antiserum of the Centers for Disease Control lacked potency when electrophoresed on the same CIE plate and against the same positive controls as were employed in tests with our own antiserum. It should be noted, however, that preparation of highest quality *P. carinii* antiserum is technologically difficult and that various degrees of success may be achieved with different lots of antibody.

With regard to accuracy, the CIE test may be from 64 to 95% accurate in tissue-documented cases (18), depending upon (i) when blood was collected with regard to the onset of acute disease, (ii) conditions under which the serum was stored and length of storage, and (iii) whether trimethoprim-sulfamethoxazole had been administered within 24 h before the specimen was drawn. Both documented and anecdotal data have shown that trimethoprim-sulfamethoxazole administration may induce the disappearance of circulating antigen as early as 24 h after beginning treatment (18; L. Pifer, D. Pifer, L. Freeman-Shade, D. Woods, J. Beattie, and P. Hanks, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 340).

With regard to serological data, all evidence suggests that

P. carinii is a ubiquitous agent that is likely present in a latent state in all mammalian species yet investigated (20). Of children aged 2 to 4 years, 80 to 90% have antibody to this organism (13, 18). In addition, nearly 80% of the lymphocytes in normal adults exhibit a positive proliferative response to *P. carinii* antigen (7).

Over 3,000 postmortems on randomly selected adults revealed that an average of 3.3% of these individuals were asymptomatically colonized with *P. carinii*, as demonstrated by the presence of *P. carinii* cysts in stained lung sections (20). In the present study, 3% of adult controls exhibited antigenemia. In cancer patients, 4.7% of adults and 5.6 to 13.3% of the children without clinical PCP had these organisms in lung tissue at postmortem (20). These values are highly consistent with the incidence of positive CIE tests in both adult and pediatric patients with cancer (14, 18). These data confirm an earlier report that cell-mediated immunity is apparently of primary importance in maintaining resistance to *P. carinii*, since substantially high antibody titers do not appear to confer clinical immunity (18).

With regard to clinical data, incidence of biopsy- or autopsy-proven *P. carinii* infection, with or without symptoms, increases in direct proportion to the compromise of immunological defenses (8). It is therefore not surprising that the incidence of antigenemia follows a similar pattern. In conclusion, the most likely interpretation of these data is that *P. carinii* is a latent commensal opportunist that may begin to increase in numbers as cell-mediated immune defenses undergo compromise. This is usually ultimately evidenced by appearance of antigen in the peripheral circulation.

That patients in group 3 (febrile with nonpulmonary infection) and 4 (afebrile with chronic lung disease) (9%) and also in group 2 (febrile with pulmonary infection) (11%) showed a threefold to nearly fourfold increase, respectively, in antigen positivity, suggests that nonspecific responses resulting from activation of immunological defenses against infection or chronic cell-mediated inflammatory processes may act to liberate *P. carinii* antigen from the lung. It is well established that pneumococcal polysaccharides are liberated from the lung into the peripheral circulation and are detectable there for as long as 2 weeks after the acute infection has been essentially resolved. Therefore, a positive CIE test may accurately reflect latent infection rendered detectable due to antigen mobilization occurring during inflammatory processes, as opposed to an acute life-threatening infection. In these patients, antigenemia is an incidental result of these events and is unlikely to indicate clinical PCP.

That CIE tests on homosexual individuals were all negative is not surprising in that all were healthy young men without any significant clinical symptomatology, despite the fact that, statistically, they belong to a higher-risk group. Acquired immune deficiency syndrome and associated clinical manifestations occur more frequently in people living in certain major metropolitan areas (4). These data show that these subjects are no more likely to exhibit *P. carinii* antigenemia than are controls.

Group 7, which had the highest percentage of antigen-positive subjects, is of greatest clinical concern. A number of these individuals were in relapse and were having difficulty with infections (ranging from urinary tract infections to sepsis), all of which provoked febrile responses. Although many had pneumonia, none developed acute PCP.

In conclusion, these data suggest that *P. carinii* is a common infectious agent that becomes clinically significant primarily when host defenses are impaired, i.e., in chronic disease or debility, therapeutic or disease-related immune suppression, immaturity, or congenital immunodeficiency. Antigenemia may occur intermittently during various disease states in the absence of positive clinical signs and should alert the clinician to subacute infection. In that eventuality, vigilant observation for development of the more commonly reported clinical manifestations of PCP (tachypnea, fever, suspicious roentgenogram, etc.) should be made. Treatment on the basis of CIE data alone does not appear warranted. Treatment does appear advisable when the history of the patient with regard to risk factors, clinical data, and CIE results concur.

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