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Detection of Antibodies to *Pneumocystis carinii* by Enzyme-Linked Immunosorbent Assay in Patients with Acquired Immunodeficiency Syndrome (AIDS)

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Introduction

Pneumocystis carinii pneumonia is the commonest opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS) (Center for Disease Control, CDC, 1983a, b), which seemed to be caused by human T-lymphotropic virus type III (HTLV-III) or known as lymphadenopathy-associated virus (LAV) (Brre-Sinoussi *et al.*, 1983; Shupbach, *et al.*, 1984). In patients with diffuse interstitial pneumonia caused by *P. carinii*, the diagnosis has been based on the histologic examination of pulmonary tissue obtained by either open lung biopsy or transbronchial biopsy (Chopra and Mohenifar, 1979; Maffhay *et al.*, 1977; Michaelis *et al.*, 1976; Quinn, 1984). Although the diagnostic value of both procedures has been clearly evident, their morbidity may be significant. Recently, serological examination by indirect immunofluorescence (IF) was reported to be a good diagnostic tool to assess the efficacy of therapy of *P. carinii* pneumonia (Tanabe *et al.*, 1985).

In the present study, an enzyme-linked immunosorbent assay (ELISA) was developed to detect serum antibodies to *P. carinii* in patients with severe immunodeficiency including AIDS patients. And the sensitivity was compared with the detection method of circulating antigen by counterimmunoelectrophoresis (CIE) routinely used in this laboratory (Tanabe *et al.*, 1985). Furthermore, we examined the antibody to HTLV-III, AIDS-associated virus, in these sera, and examined whether there was a correlation between the titers of antibodies to *P. carinii* and HTLV-III in individual cases.

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Materials and Methods

Sera: All patients studied were undergoing inpatients in a hospital at Chicago City and their serum samples were provided by Dr. N. Lord (Tolord, Chicago, U.S.A.). The patients were 21–67 years of age, 33 cases out of 55 were homosexuals and the remaining patients were heterosexual men. Thirteen of these patients were fulfilled with the diagnostic criteria for AIDS defined by CDC (1983a, b). They had Kaposi's sarcoma, pneumonia by *P. carinii*, and other opportunistic infections. Twelve patients without AIDS but with decreased responses of lymphocyte to mitogens and antigens were included in the present study. They were fulfilled with the proposed criteria for AIDS-related complex (ARC) (Quinn, 1984). Thirty-one healthy volunteers served as control. Sera from patients and

normal humans were stored at −70°C until testing.

Enzyme-linked immunosrobent assay (ELISA) for *P. carinii* antibody: Antibody titers to *P. carinii* were examined by ELISA according to the procedure as previously reported (Furuta *et al.*, 1985). Rat-derived *P. carinii* was used for the antigen in ELISA (Furuta *et al.*, 1983), and peroxidase-labeled anti-human IgG (H & L, Cappel Labs. Cochranville, Pa., U.S.A.) diluted at 1:1000 was used as conjugate. Since absorbance of the mean and the upper limit of 95% reliable range among sera from the proven non-infected at 1:40 dilution was 0.044 and 0.086, respectively, by this method, the test sera at 1:40 dilution showing the absorbance higher than 0.090 was regarded as positive.

Counterimmunoelectrophoresis (CIE) for *P. carinii* antigen: For CIE assay for antigen deteciton, undiluted human sera were reacted with anti-*P. carinii* hyperimmune rabbit serum according to the method by Pifer *et al.* (1978). CIE plates were prepared of 1% agar (Agar Noble, Difco Lab., Detroit, Mich., U.S.A.), and electrophoresis was carried out 1.6 mA/cm for 45 min in barbital buffer as previously described (Tanabe *et al.*, 1985). Positive control serum from a patient with *P. carinii* infection was provided by Dr. L. L. Pifer of the University of Tennessee, Menphis, U.S.A.

Indirect immunofluorescence (IF): For detection of antibodies to HTLV-III, the cell line HTLV-III/H9 counting 50% HTLV-III-antigen positive cells was used for IF by the method described previously (Barin *et al.*, 1985; Hayami *et al.*, 1985). For antigen preparation, aliquotes (10–20 μl) of the suspension, containing 5×10^4 cells were placed in several spots on isles slide, then air drid. They were fixed in cold acetone for 5 min and used immediately after drying, or stored at −70°C for later use. Portions of serially diluted test sera were placed on each spot of dried cell suspension, the slide was incubated at 37°C for 30 min and treated with fluorescein conjugated rabbit sera to human IgG (gamma chain specific, Dako, Gelosprup, Denmark) at 37°C for 30 min. Then, the slide was washed three times with phosphate buffered saline (PBS, pH 7.2), and mounted with glycerol-PBS. The serum showing positive reactions by IF at a dilution higher than 1:10 were regarded as positive. Results with sera from different patients and control groups were evaluated for comparison.

Results

Of 84 sera tested for *P. carinii* antibody and antigen by ELISA and CIE, respectively, 26 were positive for ELISA and 1 in 26 was positive for both tests. As shown in Table 1, when examined cases were divided into AIDS, ARC, other diseases without AIDS or ARC, and normal, their Mean O.D. values in ELISA and their S.D. were as follows: AIDS; 0.900, 0.044, ARC; 0.840, 0.038, other diseases; 0.101, 0.045, normal human; 0.046, 0.020.

The prevalence rates were 61.5, 41.4, 41.7 and 3.3% in AIDS, ARC, other diseases and normal groups, respectively. As was assumed, the high prevalence of anti-*P. carinii* antibody and high absorbance in ELISA were found in AIDS group. Circulating antigen of *P. carinii* was detected in only one patient in AIDS group of which O.D. value was as high as 0.154 by ELISA.

In order to determine the association of *P. carinii* infection with HTLV-III infection, HTLV-III antibody was examined in these sera (Table 2). Positive prevalence rates for HTLV-III antibody were 6/13 (46.2%), 11/17 (64.7%), 3/24 (12.5%) and 0/30 (0%) in AIDS, ARC, other diseases and normal groups, respectively. Thus, the prevalence of HTLV-III antibody in AIDS group was lower than those from ARC group. The same finding has been so far reported that negative or lower antibody titer against HTLV-III was observed in AIDS rather than ARC group (Barin *et al.*, 1985; Shupbach *et al.*, 1984). HTLV-III antibody was positive in 20 of 84 sera examined, and 9 of 20 were positive for both antibodies to HTLV-III and *P. carinii*. Although both anti-

Table 1 Detection of antibody to *P. carinii* by ELISA, and circulating antigen of *P. carinii* by counterimmunoelectrophoresis (CIE)

Group		ELISA positive No./ No. tested (%)	CIE positive No.
1. AIDS	total	8/13 (61.5%)	
Homo. & Hemop.		1/1	0
Homo.		5/9	1
Hete.		2/3	0
2. ARC	total	7/17 (41.1%)	
Homo.		5/11	0
Hete.		1/3	0
Hemop.		1/3	0
3. Patient without AIDS or ARC	total	10/24 (41.7%)	
Homo.		3/12	0
Hete.		7/12	0
4. Normal human	total	1/30 (3.3%)	0
5. Grand Total		26/84	1

Homo: Homosexual.
Hete: Heterosexual.
Hemop: Hemophilia.

bodies were detected in 5 of 6 sera in AIDS group, there was no significant correlation between the titers of both antibodies ($r=0.656$, $P>0.05$) in AIDS group as well as in the other groups.

Discussion

In the present study, ELISA was demonstrated to be useful for detection of antibody to *P. carinii*. Although Hofmann *et al.* (1985) was successful in detecting antibody to *P. carinii* in AIDS by IF test, antibody titers were very low, and the relationship between presence of *P. carinii* antibody and AIDS was not clear. In the present study, prevalence of *P. carinii* antibody obtained by ELISA was higher than that by IF test as reported by Hofmann *et al.* (1985), and our ELISA procedure seemed to be more sensitive. Our preveous report (Furuta *et al.*, 1985) suggested that the sensitivity of ELISA was 2 to 30 times higher than IF in mice experimentally infected with *P. carinii*.

As shown in Table 1, a high prevalence of *P. carinii* antibody was demonstrated in AIDS although they were at severe immunodeficient status. The prevalence of *P. carinii* infection in AIDS patients detected by ELISA was almost the same as those previously reported by histopathologic or other examination methods which directly demonstrate *P. carinii* cysts (CDC, 1983a, b; Chopra and Mohenifar, 1987; Maffhay *et al.*, 1977; Michaelis *et al.*, 1976). Moreover, some patients with other diseases also showed antibody to *P. carinii*. Since it has been reported that the person to person infection with *P. carinii* was found within the hospital, and subclinical *P. carinii* infection was detected in apparently normal individuals, data in the present study suggested a possibility of outbreak of *P. carinii* infection in the hospital as has been reported previously (Walzer, 1977). In control group, only one of 30 healthy individuals was positive for *P. carinii* antibody.

Table 2. Prevalence of antibodies to *P. carinii* among HTLV-III positives

Group	Antibodies	
	HTLV-III*	*P. carinii*‡
1. AIDS		
Hete	1280†	+ (0.168)§
Homo & Hemop	320	+ (0.096)
Hete	80	+ (0.165)
Homo	80	+ (0.154)
Hete	40	− (0.078)
Homo	20	+ (0.090)
		(r=0.656, P>0.05)
2. ARC		
Homo	1280	+ (0.090)
Hete	1280	− (0.044)
Hemop	1280	− (0.030)
Hete	640	− (0.058)
Hemop	320	− (0.048)
Homo	160	+ (0.095)
Homo	160	− (0.063)
Homo	160	− (0.040)
Homo	160	− (0.065)
Homo	160	− (0.077)
Homo	40	− (0.071)
3. Other diseases without AIDS or ARC		
Homo	640	− (0.047)
Homo	320	+ (0.116)
Homo	80	+ (0.098)

* Antibody titer was measured by IF.
† Reciprocal antibody titer.
‡ Antibody detection by ELISA.
§ Parenthesis means O.D. value by ELISA: Positve > 0.090.

The results in the present study that *P. carinii* antibody was detected in immunodeficient patients such as AIDS or ARC seem to be contradictory. Actually, antibodies against *P. carinii* were not detected by Maddison *et al.* (1982) and by Meuwissen *et al.* (1977) in *Pneumocystis* pneumonia patients. A serological study of 6 infants with primary immunodeficiency diseases failed to demonstrate the antibodies either (Walzer *et al.*, 1976).

Nevertheless, Maddison *et al.* (1982) could detect the antibody by their ELISA in 33% of the homosexual patients with Kaposi's sarcoma. Recent years, Hoffmann *et al.* (1985) were successful in detecting the antibody in AIDS and ARC, and Tanabe *et al.* (1985) were also in secondarily immunodeficient patients.

Reviewing those reports, Tanabe *et al.* (1985) considered that whether the antibody was detected or not might be attributed

essentially to the nature of immunodeficiency due to the underlying diseases. In fact, it was demonstrated that humoral immunity remained intact in AIDS patients even when lymphocyte proliferation was depressed (Masur *et al.*, 1981). On the contrary, the immunological state in infants with primary immunodeficiency may show deficiencies in both humoral and cellular immunity in most cases and differ from immunosuppressed patients (Walzer *et al.*, 1976).

In the present study, all of the patients were adults whose immune systems were secondarily suppressed by immunosuppressive agents such as HTLV-III virus and, therefore, belonged to the former and the antibody could be detected successfully. There is a difficulty to compare the results in reports showing unsuccessful antibody detection with those of the present study because of the different technical procedures of ELISA and in preparation of antigen.

Generally, the O.D. values of ELISA in the sera from AIDS and ARC patients were lower than those from other disease group. This may be explained by the severe deficiency of helper T cell function in these patients (Barin *et al.*, 1985; Gottlieb *et al.*, 1981; Sarngadharan *et al.*, 1984). Circulating antigen of *P. carinii* was detected in only one patient of AIDS group. In the other report (Pifer *et al.*, 1984), circulating antigen was detected in patients with various diseases as well as in normal control. The low prevalence of circulating antigen in our study might be attributable to the low sensitivity of our CIE system (Tanabe *et al.*, 1985) or the instability of circulating antigen while preserving test sera (Pifer *et al.*, 1984).

Among sera from AIDS patients, there appears to be a wide range of variation in antibody titers to HTLV-III. In present study, the prevalence of antibody positives in AIDS was lower than in ARC group. This is consistent with the findings that HTLV-III infection causes an initial lymphoid proliferation but eventually causes death of the target T lymphocytes leading to low proportion of helper T cells and a resulting lack of many helper functions including production of antibodies by B cells (Sarngadharan *et al.*, 1984). Presence of HTLV-III antibodies in AIDS group seemed to be associated with *P. carinii* infection, but no association was observed in other groups. An awareness of association should need further study of the epidemiology of *Pneumocystis* infection in HTLV-III infected persons.

Present findings suggested that *P. carinii* was infected widely and subclinically in ARC, hemophiliac patients and normal person as well as AIDS. Moreover, this study indicated the evidence that ELISA is practically valuable and highly sensitive for detecting antibody to *P. carinii* and an accurate and rapid diagnostic tool in immunocompromised hosts including AIDS.

Summary

Serodiagnosis of *Pneumocystis carinii* infection was made by demonstration of *P. carinii* antibody and circulating *P. carinii* antigen in acquired immunodeficiency syndrome (AIDS), AIDS-related syndrome complex (ARC), diseases without AIDS or ARC, and normal human groups. Antibody against *P. carinii* was detected by enzyme-linked immunosorbent assay (ELISA), and circulating antigen was detected by counterimmunoelectrophoresis (CIE). The antibodies were detected 61.5% of AIDS, 41.4% of ARC, 41.7% of other diseases, and 3.3% of normal human. Circulating antigen was detected in one patient with *P. carinii* infection. The prevalence of *P. carinii* infection revealed by ELISA in AIDS was almost the same as that reported by diagnosis by histopathologic examination. Furthermore, the antibody to HTLV-III was examined in those sera, and correlation was examined between the titers of antibodies to *P. carinii* and HTLV-III in individual cases. Although the prevalence of HTLV-III antibody was 83.3% of positive sera for *P. carinii,* there was no correlation between them ($r=0.656$, $P>0.05$).

The present findings suggest that *P. carinii* widely infects among patients with severe immunodeficiency, including AIDS. Moreover, this study indicated that ELISA was practically valuable as a highly sensitive method for detecting antibody to *P. carinii,* and is an accurate and rapid diagnostic tool in immunocompromised host.

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ELISA を用いた AIDS 患者における *Pneumocystis carinii* 抗体の検出について

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Pneumocystis carinii（Pc）抗体および血流中のPC抗原を測定することにより AIDS 患者における Pc の感染状況について調べた．また，同時にHuman T cell lymphotropic virus type-III（HTLV-III）に対する抗体についても調べた．Pc抗体はラット由来の Pc シストを抗原として ELISA により調べた。血流中の抗原はラット Pc 免疫血清を用いて対向流電気泳動法で調べた．HTLV-IIIに対する抗体は H 9 ／HTLV-III 細胞を抗原として間接蛍光抗体法で調べた．その結果，AIDS 患者の61.5%に Pc 抗体が認められ，血流中の Pc 抗原は 1 人の Pc 抗体陽性の AIDS 患者に検出された．HTLV-III 抗体は Pc 抗体陽性の AIDS 患者の46.2%に認められた．