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Serologic Response to *Pneumocystis carinii* of Seoul National University Hospital Patients

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Abstract: A total of 2,580 sera of the patients who were consulted to the serology laboratory of the Seoul National University Hospital were collected in 1990. The sera were screened by micro-ELISA to detect IgG antibody reacting with *Pneumocystis carinii* antigen. The absorbances were 0.00 to 1.41 and mean 0.27±0.253. As the positive criterion was set absorbance 0.2 or more with 70% sensitivity, total positive rate was 44.4%. Mean absorbances and positive rates were higher in children than in adults; 0.40 and 62.9% in 0 year group, 0.50 and 81.2% in 1 year group, 0.41 and 66.0% in 2-3 year group, 0.33 and 61.4% in 4-5 year group, 0.25 and 42.3% in 6-10 year group respectively. In the age groups over 11 years, the absorbances were in range of 0.16 to 0.23 and the positive rates were 26.1% to 41.5%. The present level of absorbances and positive rates could be regarded similar with those in normal Korean population. The present findings suggest that most humans are exposed to *Pneumocystis* within 2 years after birth and meet much less new antigenic challenge after 11 years in Korea.

Key words: *Pneumocystis carinii*, human serum, IgG antibody, ELISA, age, sex, hospital, Korea

INTRODUCTION

Today the number of patients who suffer from various complications of immune deficiency are increasing continuously. *Pneumocystis carinii* (Pc) is the most important opportunistic pathogen for the immune handicapped patients. More than half of AIDS patients die due to Pc pneumonia (Walzer *et al.*, 1989). The significance in medicine has required great efforts to unveil its biological nature. However, important informations on this prevailing protist such as taxonomic position, life history, *in vitro* cultivation requirements, and karyotypes are still under debates (Edman *et al.*, 1988; Cushion, 1989; Cushion *et al.*, 1988 & 1990; Watanabe *et al.*, 1989; Stringer *et al.*, 1989; Frenkel *et al.*, 1990; Hong *et al.*, 1990).

It has been proved that Pc possesses definite antigenicity in human hosts (Meuwissen *et al.*, 1977; Pifer *et al.*, 1978 & 1987; Maddison *et al.*, 1982; Tanabe *et al.*, 1985; Furuta *et al.*, 1986; Peglow *et al.*, 1990; Sinclair *et al.*, 1991). It is not so virulent that normal immune reaction can suppress its proliferation and prevent from overt Pc pneumonia. Therefore it has been speculated that Pc dwells in the lungs of most people silently. Only in occasions of immune derangement develops clinical Pc pneumonia.

At present, Pc is suspected to be a saprophyte which is transmitted by air. And most humans are exposed to its infection in early life (Meuwissen *et al.*, 1977; Pifer *et al.*, 1978; Peglow

et al., 1990). Especially, Peglow *et al.* (1990) recorded that 86% of surveyed Americans over 2 and a half years were serologically positive. Other papers recorded a little different results from Peglow *et al.* (1990), but most of the data suggested that most of healthy humans encountered its invasion already.

The frequency of serologic conversion to Pc antigens in healthy population in a community mainly depends upon the mode of transmission and number of infected organisms. If air-borne droplets were the main source of human Pc infection, hygienic standards would be very important. And thus the degree of transmissibility or endemicity should be varied by the community.

In Korea, there found a few papers on human cases of Pc pneumonia (Cheong *et al.*, 1983). Because it is known to spread over the world and to infect various mammals other than humans, it must be highly prevalent in Korea. Until now, however, no papers are available on epidemiology of Pc in Korea. Micro-ELISA is one of valuable methods that can prepare indirect evidence for prevalence of a parasitic organism. The present paper is presenting the results of micro-ELISA screening of human sera in Korea using crude extract antigen of rat Pc.

MATERIALS AND METHODS

1. Preparation of Pc antigen: The organisms were collected from the lungs of Sprague-Dawley rats which were suppressed of their immunity for 10 weeks by injecting methylprednisolone (Depomedrol®, Upjohn Inc.) subcutaneously. Details of purification of the organism were as described in Cushion *et al.* (1985) and Kim *et al.* (1987). The organisms were homogenized in PBS through a teflon coated homogenizer (Tri-R Instruments Inc., U.S.A.) and an ultrasonicator (W-385 Heat Systems-Ultrasonics Inc., U.S.A.). Supernatant of the homogenate was used after 12000 rpm centrifugation for 90 minutes at 4°C. Protein content was 5.1 mg/ml by Lowry method.

2. Collection of human sera: The sera that had been consulted to the serology laboratory of Seoul National University Hospital were collected in September to November 1990. The samples looking grossly abnormal were excluded. Their identification number, name, age and sex were filed for records, but hospital records of the individuals were not available. The sera were stored at -70°C until use. The 10 sera used for positive reference were proved to include *Pneumocystis* specific IgG antibody by Western blotting in the laboratory of Infectious Diseases Division, University of Cincinnati Medical Center, Ohio, U.S.A. (Peglow *et al.*, 1990). Dr. Cushion, M.T. and Dr. Walzer, P.D. supplied the sera.

3. Micro-ELISA: This step followed the procedure generally executed (McLaren *et al.*, 1978; Hong, 1988). The antigen was diluted 1 : 800, serum 1 : 8 and the conjugate (peroxidase conjugated anti human IgG, H & L chain specific goat sera, Cappel Co., U.S.A.) 1 : 4000. The substrate for color reaction was OPD 4 mg/10 ml. Absorbance was recorded at 490 nm with an ELISA reader (Dynatech Inc., U.S.A.).

RESULTS

The absorbances of 2,580 subjected sera were 0.00 to 1.41, mean 0.27±0.25 (Table 1). Mean absorbance was higher in the age groups 0, 1, and 2~3 years than in other groups. Peak absorbance was in the group of 1 year. The absorbances decreased continuously by 10, and showed little changes in the groups after 11 years. There found no differences by sex (Table 1).

The reference sera showed absorbances 0.07 to 0.79, mean 0.35±0.241. The cut off absorbance for positive reaction was set 0.2 or more with 70% sensitivity as the absorbances of the positive reference sera were considered (Fig. 1). Positive rate of total cases was 44.4%, male 45.1% and female 43.2%. The rate was also higher in young children groups of age 0, 1 and 2~3 years than in children over 6 or adults. The changing pattern by the age group was

Table 1. ELISA absorbance using *Pneumocystis* antigen in the subjected population by age and sex

Age Group (Year)		No. of exam.	Absorbance Range(Mean±S.D.)	Positive rates (%)
0	M	195	0.00~1.12(0.39±0.308)	62.6
	F	112	0.00~1.09(0.40±0.314)	63.4
	T	307	0.00~1.12(0.40±0.311)	62.9
1	M	97	0.00~1.10(0.51±0.299)	82.5
	F	68	0.02~1.06(0.49±0.287)	79.4
	T	165	0.00~1.10(0.50±0.294)	81.2
2~3	M	142	0.01~1.01(0.45±0.292)	72.5
	F	64	0.02~0.94(0.32±0.294)	51.6
	T	206	0.01~1.01(0.41±0.299)	66.0
4~5	M	113	0.01~0.96(0.31±0.250)	60.2
	F	76	0.04~1.06(0.36±0.268)	63.2
	T	189	0.01~1.06(0.33±0.258)	61.4
6~10	M	302	0.00~1.18(0.24±0.247)	40.1
	F	140	0.02~1.01(0.28±0.254)	47.1
	T	442	0.00~1.18(0.25±0.250)	42.3
11~20	M	241	0.00~0.93(0.19±0.220)	31.1
	F	137	0.00~0.77(0.16±0.162)	24.1
	T	378	0.00~0.93(0.18±0.201)	28.6
21~30	M	57	0.04~0.87(0.24±0.195)	43.9
	F	61	0.01~0.85(0.22±0.176)	39.3
	T	118	0.01~0.87(0.23±0.185)	41.5
31~40	M	122	0.04~0.75(0.19±0.139)	32.0
	F	59	0.02~0.72(0.17±0.122)	30.5
	T	181	0.02~0.75(0.18±0.134)	31.5
41~50	M	147	0.01~0.98(0.17±0.134)	27.2
	F	81	0.01~0.51(0.17±0.103)	27.2
	T	228	0.01~0.98(0.17±0.124)	27.2
51~60	M	141	0.04~0.61(0.17±0.115)	28.4
	F	89	0.03~0.45(0.15±0.093)	22.5
	T	230	0.03~0.61(0.16±0.107)	26.1
61~	M	81	0.02~1.41(0.21±0.221)	32.1
	F	55	0.03~0.64(0.17±0.125)	32.7
	T	136	0.02~1.41(0.20±0.189)	32.4
Total	M	1,638	0.00~1.41(0.27±0.257)	45.1
	F	942	0.00~1.09(0.26±0.245)	43.2
	T	2,580	0.01~1.41(0.27±0.253)	44.4

similar with that of mean absorbances(Fig. 2).

DISCUSSION

Important factors to be considered in interpretation of serology are the subjected population,

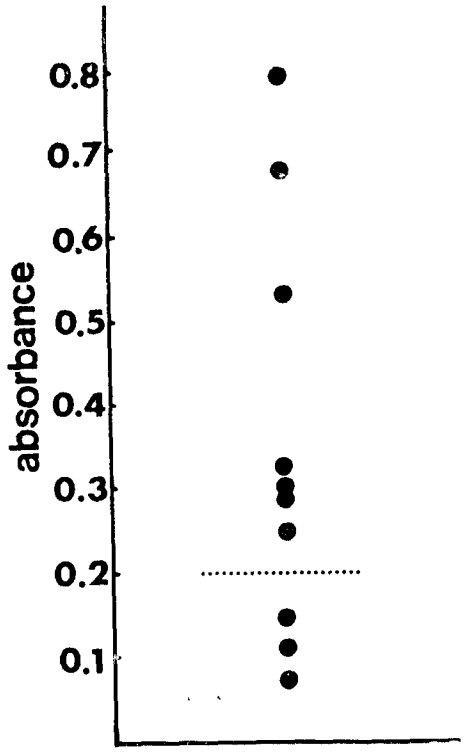

Fig. 1. Absorbance at 490nm of the 10 positive reference sera by micro-ELISA.

applied method of serology and antigen preparation. Of course the proportion of known risked population among the examined cases makes the antibody positive rate different. Maddison *et al.* (1982) observed higher absorbances in highly suspected clinical cases than in parasitologically proved ones or in healthy blood donors. Peglow *et al.* (1990) analyzed the effects of some diseases on serum level of IgG antibody. The study showed low reactivity in cases of malignancies, kidney transplants or AIDS which were of course related to immunocompetency. Such cases should have been depressed of their cellular or humoral immunity to Pc(Pifer *et al.*, 1978; Williford Pifer *et al.*, 1987; Burns *et al.*, 1990). However, the immunocompromised patients were also proved to produce detectable circulating IgG antibody in spite of their depressed immune activities(Furuta *et al.*, 1986; Williford Pifer *et al.*, 1987; Graves, 1989; Peglow *et al.*, 1990; Blumenfeld *et al.*, 1990). Summarizing ever recorded results, not all of confirmed Pc cases

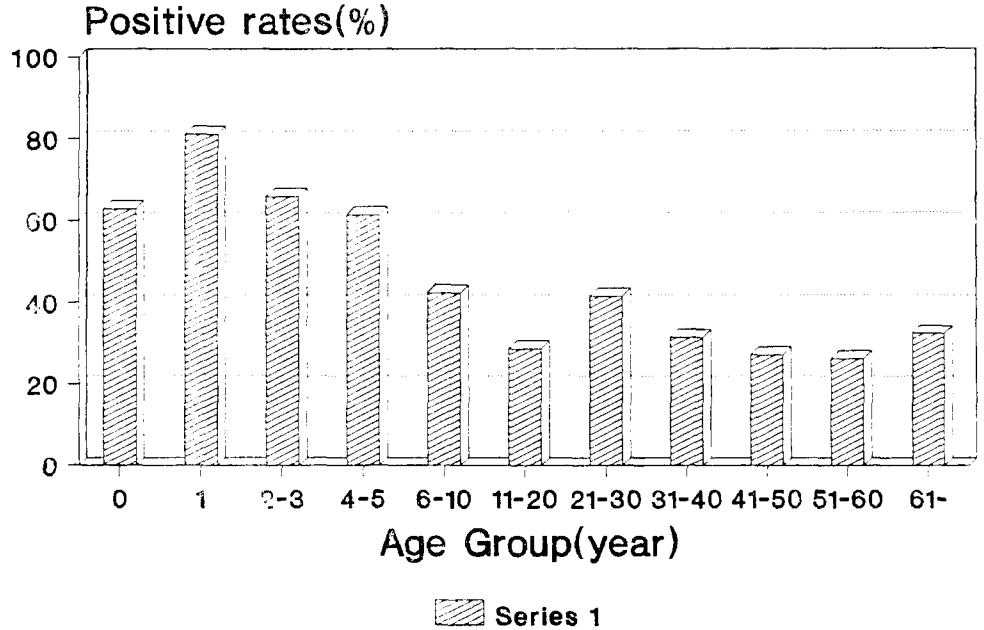

Fig. 2. The positive rates(%) by micro-ELISA by the age groups.

are seropositive and the immune compromised cases are less reactive to Pc antigen by serology. In the present study, clinical records of the subjected cases were not available unfortunately, and thus analysis of the data by individual diseased condition was impossible. However, the present serologic pattern found in 2,580 randomly selected cases in the Seoul National University Hospital could be expected not so different from that of general population because of saprophytous nature of Pc in environment. Also the hospital cares patients all over the country as the national tertiary referred center. The number of examinees also seemed to be large enough to buffer any possible biased sampling.

Another factor is the serologic method. Individual tool has limitations in feasibility, sensitivity and specificity. Same sera can make various results by the method, and this should be considered in data interpretation. Out of various methods, Western blotting(Peglow *et al.*, 1989; Blumenfeld *et al.*, 1990) and ELISA(Cho *et al.*, 1981; Furuta *et al.*, 1986; Williford Pifer *et al.*, 1987) are widely used for parasitological serology by good sensitivity and specificity, but ELISA is better for mass screening by its excellent feasibility.

It was not easy to decide the cut off absorbance in the present study. The 10 positive reference sera showed absorbances 0.07 to 0.79(Fig. 1). If the absorbance below 0.2 was regarded as negative, sensitivity of the present data could be 70%. However, it was impossible to obtain sera which were affirmed negative for Pc infection. In this context, serology on Pc infection usually reported the results without specificity.

By Western blot analysis, Pc organisms from humans revealed different antigenic determinants from that of Pc derived from rats. Most of human sera reacted to the band of 40 kDa, but partly to other bands of 66 kDa, 92 kDa, and 116 kDa(Peglow *et al.*, 1990). The sensitivity was almost doubled as all of the bands were included for analysis. Contrary to this, the rats produced IgG antibody against the bands 45 kDa, 50 kDa, and 116 kDa. The 116 kDa band was the major antigen in rats(Walzer *et al.*, 1987). In a strict sense, screening human sera should use the antigen of human Pc. Doubtlessly, better sensitivity could be expected if human Pc antigen

were used for the present assay. As most of the positive reference sera gave strong reactions, however, the Pc antigen from rats was a possible employee in the present study. In previous studies on Pc serology, only 70 to 80% of Pc pneumonia cases were positive though the sera were tested with human Pc antigen(Williford *et al.*, 1987; Peglow *et al.*, 1990). Such low frequency of antibody production might be originated from immunocompetency of the cases. Therefore, whole crude extract of rat Pc might be a good candidate antigen for the screening study supported by shared antigenic determinants with rat Pc. Maddison *et al.*(1982) checked cross reactivity of rat derived Pc antigen to confirm its specificity.

Though some limitations are inevitable as discussed above, the present serologic finding visualizes minimum evidence of Pc prevalence in Korea. Most of young children under 1 were already positive, and the positive rate reached 81.2% up until 2 years. After then, the rate was decreasing continuously by 10, and changed little in adults(Fig. 2). The antibody level in adults remained half of ever recorded. Considering low sensitivity of serology with Pc antigen as discussed above, it must be true that almost all Koreans are exposed to Pc antigen within 2 years birth. This is similar with that most of humans become serologically positive by 2 to 4 years in other countries(Pifer *et al.*, 1978; Furuta *et al.*, 1986; Peglow *et al.*, 1990). Also the finding suggests less new antigenic stimulation in adulthood than in early stage of life. This phenomenon can be read in two ways. One is the susceptibility difference between the young and the old. However, no concrete data support it. The other is low virulence of Pc, and Pc provokes its antigenicity only in subdetectable dose to a host of normal immunity. Therefore, the serology based on antibody detection has no diagnostic value of Pc pneumonia.

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REFERENCES

Blumenfeld, W., Mandrell, R.E., Jarvis, G.A. and Griffiss, J.M. (1990) Localization of host immunoglobulin G to the surface of *Pneumocystis carinii*. *Inf. Immun.*, **58**(2):456-463.

Burns, S.M., Read, J.A., Yap, P.L., and Brettle, R.P. (1990) Reduced concentrations of IgG antibodies to *Pneumocystis carinii* in HIV-infected patients during active *Pneumocystis carinii* infection and the possibility of passive immunsation. *J. Inf.*, **20**:33-39.

Cheong, S.K., Im, C.B., Ahn, D.H. and Sohn, K.C. (1983) *Pneumocystis carinii* pneumonia epidemics in an institution for adoption. *J. Korean Med. Ass.*, **26**(4):329-337(in Korean).

Cho, S.Y., Hong, S.T., Rho, Y.H., Choi, S. and Han, Y.C. (1981) Application of micro-ELISA in serodiagnosis of human paragonimiasis. *Korean J. Parasit.*, **19**(2):151-156.

Cushion, M.T. (1989) *In vitro* studies of *Pneumocystis carinii*. *J. Protozool.*, **36**(1):45-52.

Cushion, M.T. and Ebbets, D. (1990) Growth and metabolism of *Pneumocystis carinii* in axenic culture. *J. Clin. Microbiol.*, **28**(6):1385-1394.

Cushion, M.T., Ruffolo, J.J. and Walzer, P.D.(1988) Analysis of the developmental stages of *Pneumocystis carinii, in vitro*. *Lab. Inv.*, **58**(3):324-331.

Edman, J.C., Kovacs, J.A., Masur, H., Santi, D.V., Elwood, H.J. and Sogin, M.L.(1988) Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. *Nature*, **334**:519-522.

Frenkel, J.K., Barlett, M.S. and Smith, J.W. (1990) RNA homology and the reclassification of *Pneumocystis*. *Diagn. Microbiol. Infect. Dis.*, **13**:1-2.

Furuta, T., Tanabe, K., Hayami, M., Ohta, Y. and Tanaka, H.(1986) Detection of antibodies to *Pneumocystis carinii* by enzyme-linked immunosorbent assay in patients with acquired immunodeficiency syndrome(AIDS). *Jpn. J. Parasitol.*, **35**(5):419-425.

Graves, D.C.(1989) Immunological studies of *Pneumocystis carinii*. *J. Protozool.*, **36**(1):60-69.

Hong, S.T. (1988) Changes of anti-*Clonorchis sinensis* IgG antibody in serum after praziquantel treatment in human clonorchiasis. *Korean J. Parasit.*, **26** (1):1-8.

Hong, S.T., Steele, P.E., Cusion, M.T., Walzer, P.D., Stringer, S.L. and Stringer, J.R.(1990) *Pneumocystis carinii* karyotypes. *J. Clin. Microbiol.*, **28**(8):1785-1795.

Kim, C.K., Foy, J.M., Cushion, M.T., Stanforth, D., Linke, M.J., Hendrix, H.L. and Walzer, P.D. (1987) Comparison of histologic and quantitative techniques in evaluation of therapy for experimental *Pneumocystis carinii* pneumonia. *Antimicrob. Agents Chemother.*, **13**:197-201.

Maddison, S.E., Hayes, G.V., Slemenda, S.B., Norman, L.G. and Ivey, M.H.(1982) Detection of specific antibody by enzyme-linked immunosorbent assay and antigenemia by counterimmunoelectrophoresis in humans infected with *Pneumocystis carinii*. *J. Clin. Microbiol.*, **15**(6):1036-1043.

McLaren, M., Draper, C.C., Roberts, J.M., Minter-Goedbloed, E., Lighthart, G.S., Teesdale, C.H., Amin, M.A., Omer, A.H.S., Bartlett, A. and Voller, A.(1978) Studies on the enzyme linked immunosorbent assay(ELISA) test for *Schistosoma mansoni* infections. *Ann. Trop. Med., Parasit.*, **72**(3):243-253.

Meuwissen, J.H.E. Th., Tauber, I., Leeuwenberg, A.D.E.M., Beckers, P.J.A. and Sieben, M. (1977) Parasitologic and serologic observations of infection with *Pneumocystis* in humans. *J. Inf. Dis.*, **136** (1):43-49.

Peglow, S., Smulian, A.G., Linke, M.J., Pogue, C.L., Nurre, S., Crisler, J., Phair, J., Gold, J.W. M., Armstrong, D. and Walzer, P.D. (1990) Serologic responses to *Pneumocystis carinii* antigens in health and disease. *J. Inf. Dis.*, **161**:296-306.

Pifer, L.L., Hughes, W.T., Stagno S. and Woods, D. (1978) *Pneumocystis carinii* infection: Evidence for high prevalence in normal and immunosuppressed children. *Pediatrics*, **61**(1):35-41.

Sinclair, K., Wakefield, A.E., Banerji, S. and Hopkin, J.M.(1991) *Pneumocystis carinii* organisms derived from rat and human hosts are genetically distinct. *Molecul. Biochem. Parasit.*, **45**:183-184.

Stringer, S.L., Stringer, J.R., Blase, M.A., Walzer, P.D. and Cushion, M.T. (1989) *Pneumocystis carinii*: Sequence from ribosomal RNA implies a close relationship with fungi. *Exp. Parasit.*, **68**: 450-461.

Walzer, P.D., Kim, C.K. and Cushion, M.T. (1989) *Pneumocystis carinii*. From Parasitic Infections in the Compromised Host(edited by Walzer, P.D. & Genta, R.):83-178. Marcel Dekker, Inc. New York and Basel.

Walzer, P.D., Stanforth, D., Linke, M.J. and Cusion, M.T. (1987) *Pneumocystis carinii:* Immunoblotting and immunofluorescent analyses of serum antibodies during experimental rat infection and recovery. *Exp. Parasit.*, **63**:319-328.

Watanabe, J, Hori, H., Tanabe, K. and Nakamura, Y.(1989) Phylogenetic association of *Pneumocystis carinii* with the 'Rhizopoda/ Myxomycota/ Zygomycota group' indicated by compariosn of 5S ribosomal RNA sequences. *Molecul. Biochem. Parasit.*, **32**:163-168.

Williford Pifer, L.L., Niell. H.B., Langdon, S.B., Baltz, S., Clark, S.T., Edwards, C.C. and Woods, D.R.(1987) Evidence for depressed humoral immunity to *Pneumocystis carinii* in homosexual males, commercial plasma donors, and patients with acquired immunodeficiency syndrome. *J. Clin. Microbiol.*, **25**(6):991-995.