***Chlamydia pneumoniae* Infection in Children with Lower Respiratory Tract Infections**

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Summary: The incidence of antibody and the clinical features of *Chlamydia pneumoniae* (*C.pneumoniae*) infection have not been studied in children in Japan. We investigated the incidence of *C.pneumoniae* antibody in sera from 580 healthy children (including 30 umbilical cord blood samples) during the 2-year period between June 1992 and June 1994. The antibody titer was determined by a microimmunofluorescence (MIF) test by using the elementary body of *C.pneumoniae* TW-183 as the antigen. Umbilical cord blood samples were positive for the antibody in 50% of newborns tested at birth. The incidence of positivity decreased to 0% in 1-year-old children. It was still low in children up to 5 years of age and then increased rapidly in children 6 years of age or older. The positivity reached 55% in 7-year-old children and remained at this level in children older than 7 years of age. High antibody titer (IgG ≥ 512), indicating recent infection, was observed in 13 (2.2%) of the 580 children, two of whom showed no symptoms. We detected the pathogen in throat swabs by culture and capillary polymerase chain reaction (PCR), and determined IgM and IgG serum titers to *C.pneumoniae* in 130 children with lower respiratory tract infection (91 with pneumonia and 39 with bronchitis) between December 1993 and December 1994. The infection due to *C.pneumoniae* was confirmed in 10 (7.7%). Of these, 7 were boys and 3 were girls, ranging in age from 9 months to 12 years. The clinical manifestations of the infection were mild symptoms like in common cold; post-nasal discharge, hoarseness and prolonged cough were relatively characteristic. There was no significant difference in the incidence of serum positivity between the healthy children group and the patients group. The present study suggests that primary-schoolers show antibodies for *C.pneumoniae* with nearly the same frequency as adults. Mild clinical symptoms are very common in *C.pneumoniae* infections in children as in adults.

Key words: *Chlamydia pneumoniae* — children — respiratory tract infection — microimmunofluorescence test — capillary PCR

Introduction

Chlamydia pneumoniae (*C.pneumo-*

niae), TWAR strain, the third species of *Chlamydia*, is a new microorganism reported by Grayston et al. (1989). The

first isolates were TW-183 strain detected in the conjunctiva of a healthy child during a study of trachoma vaccine in Taiwan in 1965 (Grayston et al. 1986). However, the strain was not identified. Grayston et al. (1986) isolated the AR-39 strain from a student with pharyngitis at an outpatient clinic of Washington University in 1983. The biological features of this strain were the same as those of the TW-183 strain. Therefore, the TW-183 and AR-39 strains were unified as the TWAR strains. Since Marrie et al. (1987) and Grayston et al. (1989) reported these strains as important pathogens of pneumonia and bronchitis, they have attracted attention as a causative organism of respiratory infectious diseases. Subsequently, *C.pneumoniae* has come to be investigated in the Western countries. The incidence of community-acquired pneumonias in adults is believed to be 6-18% (Marrie et al. 1987; Chirgwin et al. 1991). However, such an investigation has rarely been performed in the pediatric field. Saikku et al. (1988), determining serum antibody titers in 220 children (under 5 years of age) with lower respiratory tract infection in the Philippines, reported that the incidence was 9.1%. 6.4% of the children, in whom a recent infection was suspected, showed a high antibody titer. In Japan, Kishimoto et al. (1990) found that the incidence increased rapidly in children 6 years of age or older and that the incidence in 15-year-old children was already similar to that in adults.

To clarify the incidence of *C.pneumoniae* infection in the present study, we assessed the antigen of *C. pneumoniae* by culture, capillary polymerase chain reaction (PCR), and IDEIA Chlamydia, and determined the serum antibody titers using a microimmunofluorescence (MIF) test in

children with lower respiratory tract infections and in healthy children.

Subjects and Methods

Subjects

The subjects were 130 children (age 0-15 years) with lower respiratory tract infections (91 with pneumonia and 39 with bronchitis), who were brought to the Department of Pediatrics of Kurume University Hospital or to its affiliated hospitals between December 1993 and December 1994. The normal control group consisted of 550 healthy children in the same age range (260 boys and 290 girls), who were brought to the above-mentioned facilities during the 2-year period between June 1992 and June 1994. The umbilical cord blood samples from 30 pregnant women were used for determination of antibody titer at birth. Informed consent was obtained in all cases from parents or guardians.

Methods

Determination of serum antibody titers and diagnostic criteria: Collected sera (0.5 ml) were stored at -70°C before use. The MIF test, the indirect fluorescent antibody technique using the elementary body (EB) of *Chlamydia* species as an antigen, was used for the determination of *Chlamydia* serum antibody titers, and IgM and IgG antibody titers were determined (Wang et al. 1970). The antigens used were EBs of L_2 strains of *C. trachomatis* (Washington Research Foundation, Seattle), budgerigar strains of *C. psittaci* (from Dr. Yasuo Kanamoto, Hiroshima Prefectural Institute of Public Health, Japan), and TW-183 strains of *C.*

pneumoniae (Washington Research Foundation, Seattle). The antigens were mixed with an equal amount of 3% normal yolk sac suspension and placed with a pen point in the wells of a microslide glass for fluorescent detection (Matsunami, Japan). The slide was air-dried and fixed in acetone for 15 min at room temperature. Tested serum (25 μ l) was subjected to two-fold stepwise dilution with phosphate buffered saline (PBS, pH 7.4) (IATRON, Tokyo, Japan) and reacted with the antigens in wells at 37 °C for 30 min in a moist chamber. After incubation, the slide was rinsed four times with PBS (pH 7.4) and twice with distilled water. After air-drying, the sample was reacted with fluorescein isothiocyanate (FITC)-labelled goat anti-human IgM/IgG (Cappel Inc., USA) and PBS (pH 7.4) containing 0.04% Evans blue for counterstaining. The sample was reacted at 37 °C for 30 min in a moist chamber and air-dried after rinsing. The samples were observed with a fluorescent microscope (Nikon, Japan) at 400 $\times$ after mounting in glycerol. The maximum dilution, at which the fluores-

cence specific to EB could be observed, was defined as the antibody titer (Fig. 1). An IgG antibody titer of ≥ 32 was regarded as positive for *C. pneumoniae* antibody. The diagnosis of recent *C. pneumoniae* infection was based on the following criteria (Grayston et al. 1989): (1) IgM antibody titer ≥ 16 , (2) a four-fold or greater titer change between paired sera, (3) IgG antibody titer ≥ 512 .

PCR: The nasopharynx of patients was wiped with a Dacron swab (DAKO DIAGNOSTICS LTD., USA). The swab was stored in 1 ml of transport medium containing a sucrose-phosphate-glutamate (SPG) buffer to avoid drying. The collected specimens were left to stand at 4 °C for 1-4 hs, then stored at -70 °C. 500 μ l of specimen was centrifuged at 15,000 rpm for 30 min, and DNA was extracted from the sediment by means of IsoQuick™ (MICRO PROBE Co., USA). The primers used were CHP-1 and CHP-2, which amplify the 437-base pair (bp) within *C. pneumoniae*-specific 474-bp *Pst*I restriction fragment reported by Campbell et al. (1992); CHP-1: GTTGTTCATGAAGGCCTACT,

Fig. 1. Fluorescence microscopic findings by MIF test showing the results of antibody-positive (A) and negative (B). ($\times 400$)

CHP-2: TGCATAACCTACGGTGTGTT. The PCR solution was prepared with the method of Wittwer et al. (1991); 10 μ l of 10 $\times$ buffer, 80 μ l of distilled water, and 10 μ l of 10 $\times$ dNTP were mixed to prepare the PCR buffer, and 15 μ l of the buffer was used. The capillary PCR Taq polymerase used was 1 μ l of a mixture of 1.5 μ l of Taq polymerase (TAKARA, Japan) and 6 μ l of diluent buffer. The capillary primer used was prepared by mixing together 1 μ l of a mixture of CHP-1 (5 μ l) and distilled water (15 μ l) with 1 μ l of a mixture of CHP-2 (5 μ l) and distilled water (12.5 μ l). 2 μ l of sample was added to the capillary primer to a volume of 20 μ l. The reaction solution was transferred to a glass capillary tube (Idaho-Technology Inc., USA), and the following reactions were carried out by capillary PCR amplifier (Idaho-Technology Inc., USA): (1) denaturation, 94 $^{\circ}$ C for 3 sec; (2) annealing, 55 $^{\circ}$ C for 1 sec; (3) extension, 75 $^{\circ}$ C for 15 sec. The reactions (1) through (3) were regarded as one cycle, and 50 cycles were carried out. The PCR prod-

ucts were subjected to 2% agarose gel electrophoresis, were observed under UV at 260nm and were photographed after staining with ethidium bromide (Fig. 2).

Culture: For the culture of *C. pneumoniae*, the nasopharyngeal specimen obtained with the Dacron swab (DAKO DIAGNOSTICS LTD., USA) was immediately placed in 1ml of SPG with 10% fetal bovine serum (FBS) at 4 $^{\circ}$ C for 1-4 hs and stored at -70 $^{\circ}$ C. Twenty specimens that showed high antibody titers on serological study were subsequently shipped to Dr. Hammerschlag at the State University of New York Health Science Center. During transport they were frozen at -20 $^{\circ}$ C in dry ice. Cultures were performed by inoculating 200 μ l of each specimen in cycloheximide-treated HEP-2 cells grown in 96-well microtiter plates (Roblin et al. 1992). All specimens were passaged once, and isolates were identified as chlamydiae with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostic, Chaska, Minn, USA). Identification was confirmed with a *C. pneumoniae*-specific monoclonal antibody (Washington Research Foundation, Seattle). Isolates were also stained by anti-major outer membrane protein and *C. trachomatis*-specific monoclonal antisera (Syva, Palo Alto, Calif, USA) to identify *C. trachomatis*.

Enzyme antibody technique: The IDEIA Chlamydia (DAKO DIAGNOSTIC LTD., USA) kit (IDEIA) was used. After reaction according to the manufacture's instructions, absorbance at 486nm was determined with ELISA ANALYZER ETY-96 (Toyosokki Co., Ltd., Tokyo, Japan).

Statistical analysis: A chi-square analysis

Fig. 2. Detection of *Chlamydia pneumoniae* DNA by capillary PCR and gel electrophoresis.

M: molecular weight markers; P: positive control (*C. pneumoniae* TW-183); N: negative control; lane 1-7: clinical specimens.

The expected PCR products (437-bp) are visible in lanes 1, 2, 6 and 7.

was used to determine statistical significance.

Results

Incidence of C.pneumoniae infection in pediatric patients with lower respiratory tract infection

The incidence of *C. pneumoniae* antibodies and/or antigen according to age in 130 patients with lower respiratory

Fig. 3. Prevalence rate to *C. pneumoniae* according to age in 130 patients with lower respiratory tract infection.

tract infection is shown in Fig. 3. The rate was 20% in 0-year-old patients and 0% in 1-year-old patients. The rate gradually increased in patients aged 2 years or over, reaching 42.9% in 6-year-old patients. In patients over 6 years of age, the rate remained at approximately 50%. Ten patients were diagnosed by serological testing using MIF test and positive for either PCR or culture methods for isolating *C.pneumoniae* in the 130 patients. The 10 patients were 4 (4.4%) of the 91 patients with pneumonia and 6 (15.4%) of the 39 patients with bronchitis.

Mycoplasma pneumoniae was responsible for lower respiratory infection in 22 (16.9%) of 130 patients; in 16 (17.6%) of 91 pneumonia patients and in 6 (15.4%) of 39 bronchitis patients. Other possible etiologic pathogens for lower respiratory tract infections were not tested.

The characteristics of 10 patients due to *C.pneumoniae*, 7 boys and 3 girls, are shown in Table 1. The age ranged

TABLE 1.

Characteristics of 10 patients with Chlamydia pneumoniae lower respiratory tract infection

case	sex	age	disease	PCR	culture	IDEIA	IgM	IgG (A)	IgG (C)	WBC (μ l)	CRP (mg/dl)	ESR (mm/h)	CA
1	F	9m	Bron	+	-	+	16	64	256	11,400	0.2	3	512
2	F	2y1m	Bron	+	-	-	32	<8	128	8,900	0.1	24	32
3	M	3y1m	Pneu	+	-	-	64	<8	128	17,800	1.0	51	256
4	F	3y9m	Pneu	+	-	-	<8	32	512	9,000	2.7	50	512
5	M	4y8m	Bron	+	-	-	<8	32	128	6,300	0.4	4	128
6	M	5y6m	Bron	+	-	-	<8	32	128	7,100	0.2	N.D.	16
7	M	5y8m	Pneu	+	-	-	16	64	256	10,300	3.5	55	512
8	M	5y1m	Bron	+	-	-	<8	256	512	6,900	0.2	33	16
9	M	9y5m	Bron	+	-	-	<8	1,024	1,024	8,600	0.2	23	512
10	M	12y4m	Pneu	N.D.	+	-	<8	1,024	1,024	7,800	0.3	5	32

Bron: acute bronchitis; Pneu: pneumonia; PCR: polymerase chain reaction; +: positive; -: negative; N.D.: not done; IgM: *C.pneumoniae* IgM; IgG (A): *C.pneumoniae* IgG (acute); IgG (C): *C.pneumoniae* IgG (convalescent); CA: cold agglutination test

from 9 months to 12 years old and half of them were under 5 years old. *C. pneumoniae* was detected by PCR in all 9 examined cases. One case who was not examined by PCR was positive by culture method. Only one case of 10 examined was positive by IDEIA. In laboratory tests, there were few cases who showed leukocytosis and positive C-reactive protein (CRP), but the erythrocyte sedimentation rate (ESR) was elevated in 6 cases. The cold agglutination test was elevated in 6 cases, of whom 4 cases showed high titer (≥ 512). As for serum antibodies to *C. pneumoniae*, 4 cases were positive for IgM antibody in acute sera with the range of 16-64. IgG antibody in these 10 cases ranged from

128 to 1,024.

The clinical symptoms of 10 patients is shown in Table 2. There were 4 cases of pneumonia. One of them was thought to be a mixed infection with *Mycoplasma pneumoniae* diagnosed serologically. The remaining 6 cases were bronchitis. One patient with pneumonia had to be admitted to the hospital. Cough was observed as one of the initial symptoms in all 10 patients and 8 had productive coughs. Nine were afebrile or had febricula. The remaining patient had fever greater than 38 °C. Rales were heard in 6 on auscultation. Other symptoms were post-nasal discharge that persisted for a long period (4-8 weeks) in 4 and hoarseness in 3 patients. Sinusitis was

TABLE 2.

Clinical symptoms of 10 patients with Chlamydia pneumoniae lower respiratory tract infection

	n (%)		n (%)
1) Sex		6) Auscultation	
Male	7 (70)	Rale	6 (60)
Female	3 (30)	(Wheezing)	3 (30)
2) Age (9 months to 12 years)		7) Other symptoms	
<5 years	5 (50)	Post-nasal discharge	4 (40)
≥ 5 years	5 (50)	Hoarseness	3 (30)
3) Diagnosis		Pharyngalgia	2 (20)
Bronchitis	6 (60)	Lymph-nodes swelling	1 (10)
*Pneumonia	4 (40)	Chest pain	1 (10)
4) Admission		8) Complications	
No	9 (90)	Sinusitis	2 (20)
Yes	1 (10)	Conjunctivitis	2 (20)
5) Initial symptoms		Otitis media	1 (10)
Productive cough	8 (80)	Iritis	1 (10)
Non-productive cough	2 (20)	9) Period of visiting clinics after onset	
Nasal discharge (obstruction)	6 (60)	<1 week	5 (50)
Fever ($>37^{\circ}\text{C}$)	5 (50)	<1 month	3 (30)
Fever ($\geq 38^{\circ}\text{C}$)	1 (10)	≥ 1 month	2 (20)
Joint, Muscle pain	1 (10)		
Ophthalmalgia	1 (10)		

*One was a mixed infection with *Mycoplasma pneumoniae*.

TABLE 3.
Incidence of seropositivity to *Chlamydia pneumoniae* according to age and sex in healthy children

Age (year)	no. of samples			no. of positive (%)		
	♂	♀	total	♂	♀	total
Cord blood			30	15 (50)		15 (50)
≤6 Mo	10	4	14	1 (10)	1 (25)	2 (14)
7 Mo~1	6	6	12	0 (0)	1 (17)	1 (8)
1	10	10	20	0 (0)	0 (0)	0 (0)
2	10	10	20	2 (20)	1 (10)	3 (15)
3	23	15	38	0 (0)	2 (13)	2 (5)
4	40	27	67	4 (10)	5 (19)	9 (13)
5	45	41	86	8 (18)	3 (7)	11 (13)
6	20	24	44	5 (25)	6 (25)	11 (25)
7	7	13	20	4 (57)	7 (54)	11 (55)
8	8	21	29	6 (75)	7 (33)	13 (45)
9	9	17	26	4 (44)	7 (41)	11 (42)
10	11	19	30	5 (46)	10 (53)	15 (50)
11	12	17	29	6 (50)	7 (41)	13 (45)
12	17	23	40	7 (41)	9 (39)	16 (40)
13	8	17	25	3 (38)	8 (47)	11 (44)
14	11	14	25	5 (46)	6 (43)	11 (44)
15	13	12	25	5 (39)	6 (50)	11 (44)
Total	260	290	550	65 (25)	86 (30)	151 (27)

antibody titers. The pharyngeal swab in the sister was also positive for IDEIA.

Incidence of serum antibodies against C.pneumoniae according to age in healthy children

Table 3 shows the incidence of seropositivity for *C.pneumoniae* according to age and sex. The umbilical cord blood samples from 15 (50%) of the 30 cases showed IgG antibodies. Thereafter the incidence gradually decreased and was 0% in the 1-year-old children. The incidence stayed at this low level in the children aged under 5 years but increased rapidly in those older than 6 years. More than half of the 7-year-old children (11/20 or 55%) possessed the antibodies.

There was no change in the incidence, approximately 40-50%, in children over 7 years of age (Fig. 6). There was no sexual difference in serum antibody incidence (Fig. 7).

There was no significant difference in the incidence according to age between the patients and the healthy children.

The incidence for *C.trachomatis* was 30% in the umbilical cord blood samples, but thereafter, in childhood, the incidence decreased.

The incidence for *C.psittaci* was also low, 0.86% (5/580), in all ages.

An IgG antibody titer of ≥ 512 , which has been regarded as indicating recent infection, was observed in 13 (2.2%) of the 580 subjects (including 30 cord blood

Fig. 6. Prevalence rate to *C.pneumoniae* according to age in healthy children.

Fig. 7. Prevalence rate to *C.pneumoniae* according to age and sex in healthy children.

Fig. 8. Age distribution of cases with high *C.pneumoniae* antibody titer determined by MIF test.

samples). Their ages ranged from 2 to 10 years (Fig. 8). The medical history for the previous 3 months in 8 of 13 children was taken. Six of the 8 children had recently been brought to private clinics because of cough, nasal discharge and febricula. None of them had to be admitted to the hospital under a diagnosis of pneumonia or bronchitis and two children had no symptoms.

Discussion

C. pneumoniae, the new third species of *Chlamydia* passed from human to human, was given this name by Grayston et al. (1989). Recent surveys in Europe and the United States have found that the serum of over 50% of adults have IgG antibodies to *C.pneumoniae* as determined by the MIF test, suggesting that infection by *C.pneumoniae* is common and frequently subclinical (Freidank et al. 1993; Kern et al. 1993). In these studies in Western countries there have been only a few reports on the infection in children. Surveys of the incidence of *C.pneumoniae* infection according to age in Denmark and in Seattle showed that the rate was low in infants, increased rapidly in teenagers, and continued to increase until middle age. Saikku et al. (1988) determined the *C.pneumoniae* antibody titer in 220 children with acute lower respiratory tract infection who were under 5 years of age, in the Philippines, and reported that positivity increased with age: the serum was positive in 31% of 4-year-old children. In this age group 14 children (6.4%) showed an antibody titer indicating acute infection. The investigators assumed that the

initial infection with *C.pneumoniae* occurs at a younger age in the tropics than in Northern Europe. In Japan, Kishimoto (1990) investigated the incidence of *C.pneumoniae* antibodies according to age and reported that the incidence increased more rapidly in children aged 6 years or over in comparison with Western countries and reached a value of at least 60% in children aged under 15 years. However, Kishimoto's method of determining the antibody titer to *C.pneumoniae* might have been inaccurate due to cross-reactivity with *C.trachomatis* or *C.psittaci*, because they used the microplate immunofluorescence antibody technique (MFA) which uses inclusion bodies containing reticulate body (RB) as the antigen. The MIF test we employed, which uses individual EB as the antigen, is the most reliable for the determination of specific antibody titers to various species of *Chlamydia*. Ouchi et al. (1991) investigated the incidence of individual antibodies according to age in Japan by using three specific types of EB of *Chlamydia* as antigens. Ouchi et al. determined the incidence of antibodies for each species of *Chlamydia* by MIF test in cord blood samples, sera from patients aged 7 months or over who had been brought to hospital, and sera from healthy adults. The study showed that the incidence of the *C.pneumoniae* antibodies increased rapidly in 4- to 7-year-old children, and that 44% of the 8- to 11-year-old children already possessed the antibody. The incidence was approximately 50% in the age group over 12 years. Data in healthy children have not been reported.

In the present study, we determined

the antibody titer for *C.pneumoniae* and other *Chlamydia* species (*C.trachomatis* and *C.psittaci*) by MIF test in 30 umbilical cord blood samples and in sera from 550 healthy children. We also investigated changes in the incidence of antibodies according to age. We also assessed the presence of antigen by culture or PCR method and determined antibody titers in paired sera by MIF test in pediatric patients with lower respiratory tract infection.

The survey of the *C.pneumoniae* incidence according to age in healthy children revealed that 50% of the cord blood samples had IgG antibodies. None of the samples showed elevated IgM antibody or an IgG titer ≥ 512 . The incidence decreased and reached 0% in the one-year-old children. The changes in IgG antibody titer in early infancy can be attributed to the transient presence of antibodies of maternal origin. The incidence remained low in children aged under 5 years, but increased rapidly in children aged over 6 years, reaching 55% in 7-year-old children. The incidence was 40-50% in children aged over 7 years, showing no change with age. The present results differ from the results of the surveys in Seattle and Denmark (Grayston et al. 1990) in which the incidence was elevated in younger children. In contrast, Wang and Grayston (1986) reported that the incidence in childhood in Taiwan was 41%. *C.pneumoniae* might be considered to have a higher rate of invasion in Asia than in Western countries. Because the population density in Asia is much higher than in Western countries, Saikku et al. (1988) proposed that the incidence may be related to population density.

Regarding the high antibody titers to

C.pneumoniae in healthy children in our study, *C.pneumoniae* infection is likely to produce mild symptoms like common cold or to be subclinical in most cases.

The incidence of *C.pneumoniae* in pediatric lower respiratory tract infection was 7.7% (10/130), while the incidence of respiratory tract infection by *Mycoplasma pneumoniae* was 16.9% (22/130). The frequency of involvement of *C.pneumoniae* in bronchitis was the same as that of *Mycoplasma pneumoniae*.

C.pneumoniae outbreaks were observed in group living conditions (Saikku et al. 1985; Kleemola et al. 1988; Grayston et al. 1989) and in Japan, familial infection has been reported by Yamazaki et al. (1990). The incubation period of a *C.pneumoniae* infection is thought to be approximately 3 weeks (Grayston, 1994) and the same interval was observed in our intrafamilial infections.

As for clinical manifestations, Grayston et al. (1990) reported that pharyngalgia was frequently present in *C.pneumoniae* infection with low incidence of fever and significantly high incidence of hoarseness. Hospital visits were delayed because of the mild initial symptoms. Thom and Grayston (1991) reported that sinus tenderness on percussion was observed in 36% of *C.pneumoniae* infection and about 5% of them had sinusitis. Soejima et al. (1994), investigating adult patients with *C.pneumoniae* pneumonia, reported that the incidence of non-productive cough was high. Prolonged cough, hoarseness and post-nasal discharge are considered characteristic in previous reports and this study. It is difficult to diagnose *C.pneumoniae* infection by only clinical symptoms.

According to the serological diagnosis by MIF test, IgM antibody appeared about 3 weeks after the onset of symptoms and IgG was elevated 6-8 weeks after the onset. IgM hardly appeared in reinfection and IgG titer became high 1-2 weeks after the onset of symptoms (Grayston et al. 1986). In the present study IgG antibody titer was greater than 512 in 4 of 10 patients. Four patients were thought to be initial infection as the IgG antibody titer remained at a maximum 256 even in the convalescence stage.

In cultures, the organism was isolated in only one of our 10 patients. HL cells have been considered highly sensitive as culture cells for initial isolation of *C.pneumoniae* (Cles and Stamm, 1990), but Hep-2 cells have been commonly used in cultures since Roblin et al. (1992) reported that Hep-2 cells are more sensitive than HL cells. However, the actual isolation is not easy and attempts to isolate *C.pneumoniae* from many patients in whom infection was strongly suspected have been successful in only a limited number of institutions. Probably because during the long time period between specimen collection and the start of culture, *C.pneumoniae* might have been inactivated, or because of the low number of *C.pneumoniae* itself. Kuo et al. (1988) reported that *C.pneumoniae* is inactivated by acute storage at -70°C in SPG buffer after specimen collection and is rapidly inactivated even at room temperature. For the time being, it is considered to be important that *C.pneumoniae* should be stored at 4°C for 1-4 hs soon after specimen collection and subsequently frozen at -70°C . Roblin et al. (1992) recommended the addition of 10% FBS to the SPG buffers and nasophar-

ynx specimens in throat swabs.

Capillary PCR, which was performed in the present study, is 1,000 times more sensitive than conventional PCR in the detection of *C.pneumoniae* DNA (Kawayama, 1993). Furthermore it takes only a short time, about 30 min, for the reaction. *C.pneumoniae*-specific DNA was detected by this technique in all patients in whom cultures were successful except one.

IDEIA, originally used for the detection of *C.trachomatis* antigen and *C.pneumoniae* antigen, can be used although there is a difference in sensitivity between the two species (Kanamoto, 1992). Only one patient was positive in our 10 patients. However, the amount of antigen in the specimens tested might have been small. The kit is therefore of limited usefulness for general screening.

The present study revealed that *C.pneumoniae* infection is common in Japan not only in adults but also in children. The organism has recently been reported as a cause of ischemic heart disease, and in fact, some reports describe its isolation from the coronary artery lesion (Kuo et al. 1993). In addition, we found a boy with iritis due to *C.pneumoniae* (Yamada et al. 1994). Thus *C.pneumoniae* infections present a various degree of correlation with certain diseases. Further clinical investigations would be required to establish the incidence of *C.pneumoniae* infections in childhood.

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