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Prevalence of Antibody to *Chlamydia pneumoniae* TWAR in Japan

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***Chlamydia pneumoniae* TWAR is a newly recognized *Chlamydia* species that is a pathogen of respiratory tract infection. To clarify the endemic status of *C. pneumoniae* in Japan, we evaluated the incidence of *C. pneumoniae* antibody in 1,330 serum samples (660 from outpatients, 600 from normal individuals, and 70 from cord blood). The antibody titer was determined by a microimmunofluorescence test by using the elementary body of *C. pneumoniae* TW-183 as the antigen. Immunoglobulin G antibody titers of 1:32 or higher were regarded as evidence of past infection. The detection rate of *C. pneumoniae* antibody rapidly increased in subjects between the ages of 4 and 7 years, reached 44% in subjects between the ages of 8 and 11 years, and was about 50% in older subjects. The rate did not differ between healthy subjects and outpatients. These results suggest that *C. pneumoniae* infection is highly endemic in Japan as it is in Western countries. However, the antibody prevalence was high in the low age groups in Japan compared with that in Western countries.**

Chlamydia pneumoniae was recently recognized as the third *Chlamydia* species (5), in addition to *Chlamydia trachomatis*, which causes trachoma, sexually transmitted diseases, and neonatal and infantile pneumonia and conjunctivitis, and *Chlamydia psittaci*, which causes psittacosis. *C. pneumoniae* was initially regarded as a subspecies of *C. psittaci* (6, 10, 16) but was classified in 1989 as a new *Chlamydia* species (5) on the basis of the characteristic morphology of the elementary body (3), results of evaluation with monoclonal antibody, and DNA homology (2).

Since *C. pneumoniae* has been reported (6) to cause pneumonia and bronchitis, this organism has attracted attention as a pathogen of respiratory tract infections (7, 9, 11–13, 15). However, little is known about its infection route or clinical picture (1, 8, 17).

We evaluated the prevalence of *C. pneumoniae* antibody in healthy subjects and outpatients in Japan.

Specimens. Between February and December 1988, serum samples were obtained from 660 patients (aged 7 months to 84 years) and 70 umbilical cords at the National Kure Hospital in Hiroshima Prefecture. In 1988 and 1989, serum samples were obtained from 600 healthy individuals living in Hiroshima. Most of the patients were outpatients. The serum samples were stored at −80°C until measurement.

Serological test. A microimmunofluorescence test was used to measure chlamydial antibodies (19). *C. pneumoniae* TWAR (TW-183) was provided by the Washington Research Foundation, Seattle, Wash. The antigens used were formalinized elementary bodies of *C. pneumoniae* (TW-183), *C. trachomatis* immunotypes BED, CJHI, and GFK, and *C. psittaci*. Immunoglobulin (Ig) G chlamydial antibodies were measured by using goat fluorescein isothiocyanate-conjugated anti-human IgG (Cappel Laboratories, Malvern, Pa.). Serological diagnosis of a previous infection was made when IgG antibody titers were 1:32 or higher. When high antibody titers were observed in outpatients, we examined whether respiratory tract infections had occurred within 3 months of serum collection. This was done by investigating medical charts and taking past histories by telephone.

Statistical analysis. The data obtained were analyzed by the χ^2 test.

***C. pneumoniae* antibody prevalence.** Table 1 shows the age-related incidence of *C. pneumoniae* antibody. Among the outpatients (group 1), the antibody detection rate rapidly increased in subjects between the ages of 4 and 7 years, reached 44% in subjects between the ages of 8 and 11 years, and was about 50% in older subjects. The antibody was positive in 51% of the samples from cord blood; this rate was similar to that in the maternal age group (20 to 30 years old). In healthy subjects aged 16 years or older (group 2), the antibody was present in 52%. Antibody to *C. pneumoniae* in subjects less than 20 years old was present in 98 (35.5%) of 276 males and 84 (37.5%) of 224 females. In subjects over 20 years old, *C. pneumoniae* antibody was present in 193 (52.2%) of 370 males and 175 (44.9%) of 390 females. Therefore, no relationship was found between gender and incidence of *C. pneumoniae* antibody.

Table 2 shows the distribution of *C. pneumoniae* antibody titers of 1:32 or higher in group 1. Antibody titers of 1:256 or higher were observed in 11% of the outpatients that tested positive for antibody.

Recent respiratory tract infections in outpatients with high *C. pneumoniae* antibody titers. We were able to take the medical history for the previous 3 months in 9 of 11 outpatients who had a *C. pneumoniae* titer of 1:521 or higher, which is considered to indicate a recent infection (8). Of these nine outpatients, three had developed pneumonia. Two had suffered from *Mycoplasma pneumoniae* infection and the other had had pneumonia of unknown origin (including *C. pneumoniae*) during the previous 3-month period. Three of the remaining six outpatients had developed cold-like symptoms and tonsillitis, but the other three had been healthy.

Recently, Grayston et al. (8) suggested, from their long experience with *C. pneumoniae* infection, that antibody

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TABLE 1. Presence of *C. pneumoniae* antibody by age group in Hiroshima

Age	Group 1[a]		Group 2[b]	
	No. of serum samples	No. positive[c] (%)	No. of serum samples	No. positive[c] (%)
Newborn (cord blood)	70	36 (51)		
6 mo–3 yr	119	5 (4)		
4–7 yr	82	11 (13)		
8–11 yr	62	27 (44)		
12–15 yr	59	27 (46)		
16–19 yr	78	42 (54)	100	59 (59)
20–29 yr	60	27 (45)	100	50 (50)
30–39 yr	50	23 (46)	100	43 (43)
40–49 yr	50	25 (50)	100	48 (48)
50–59 yr	50	22 (44)	100	56 (56)
≥60 yr	50	24 (48)	100	61 (61)
Total (avg)	730	269 (37)	600	310 (52)

[a] Group 1 consisted of 660 serum samples from patients and 70 serum samples from cord blood at National Kure Hospital.
[b] Group 2 consisted of 300 serum samples from healthy subjects and 300 serum samples from blood donors in Hiroshima.
[c] A titer of 1:32 or higher in the IgG serum fraction, measured by the microimmunofluorescence test, was considered positive.

titers of 1:16 or higher indicate past infection. However, *C. pneumoniae* antibody titers of 1:32 or higher have been reported in several populations around the world (14, 18), so we considered a serum titer of 1:32 or higher as positive in order to compare our results with earlier studies. The detection rate of *C. pneumoniae* antibody increased rapidly in subjects between the ages of 4 and 7 years, and 44% of subjects aged 8 to 11 years already showed the antibody. In subjects aged 12 years or older, the antibody detection rate was about 50%. The detection rate did not differ between outpatients and healthy controls. The rate in the adults was similar to that (45%) in 196 male patients with sexually transmitted diseases in Japan (18). The prevalence of *C. pneumoniae* antibody began to increase in subjects between the ages of 4 and 7 years in several limited populations in Western countries (4, 18). Compared with our subjects, this increase was more gradual and the rate continued to increase even in adults, reaching about 50% in subjects between the ages of 30 and 40 years. The rapid increase in the antibody detection rate in children in Japan, which is as developed as and more densely populated than Western countries in the present study and in other studies in Asia (4, 15, 18), suggests that *C. pneumoniae* is more endemic in Asia than in Western countries. Saikku et al. (15) suggested that the prevalence of *C. pneumoniae* is associated with population density.

TABLE 2. Distribution of *C. pneumoniae* antibody titer in outpatients

Antibody titer[a]	No. positive
1:32	106
1:64	84
1:128	49
1:256	19
1:512	10
1:1,024	1

[a] Antibody titer, measured by microimmunofluorescence, in the positive patients in group 1 shown in Table 1.

As a diagnostic criterion for recent infection, Grayston et al. used a *C. pneumoniae* IgG antibody titer of 1:512 or higher (8). However, our investigation of the medical history for the 3 months preceding serum collection outpatients with an antibody titer of 1:512 or higher found diseases such as pneumonia in only some of them. Actually, two of the outpatients had suffered from pneumonia caused by *M. pneumoniae*. Thus, caution is needed in diagnosing pneumonia in patients with a high *C. pneumoniae* IgG antibody titer. A fourfold or greater increase in the IgG antibody titer in paired serum samples or confirmation of IgM antibody seems to be necessary. Although the association of *C. pneumoniae* infection with pneumonia and bronchitis has been suggested, the low incidence of these diseases in patients with high antibody titers suggests only that mild coldlike symptoms and mild reinfection frequently occur (8).

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