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Chlamydia pneumoniae infection in adult patients with persistent cough

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Chlamydia pneumoniae is a frequent causative agent of acute respiratory disease. To assess whether *C. pneumoniae* plays a role in persistent cough, the prevalence of *C. pneumoniae* infection in adult patients with persistent cough was investigated. Nasopharyngeal swabs and serology samples from 366 adult patients with a persistent cough lasting in excess of 2 weeks and 106 control subjects were analysed for bacterial isolation and by PCR. *C. pneumoniae* was isolated from two patients and from none of the controls and was detected by PCR in 20 patients and one control. Serological evidence of acute *C. pneumoniae* infection was present in 24 patients but in none of the controls. Of these 20 patients who were positive by culture and/or PCR, three were still positive by PCR after 2 weeks of treatment with clarithromycin and symptoms either continued or relapsed. However, when patients were treated with clarithromycin for 5–6 weeks, their symptoms disappeared completely and the results of their cultures and/or PCR for *C. pneumoniae* became negative. These data suggest that *C. pneumoniae* infection may cause persistent cough in adults. Furthermore, these data also indicate that it may be necessary to eradicate the organism when *C. pneumoniae* is detected by culture and/or PCR in patients with persistent cough.

INTRODUCTION

Chlamydia pneumoniae has been shown to cause both epidemic and endemic respiratory tract infections in many areas of the world (Grayston *et al.*, 1990; Kuo *et al.*, 1995). It is a common cause of lower and upper respiratory illnesses, including pneumonia, bronchitis, pharyngitis and sinusitis, in both children and adults (Grayston *et al.*, 1990; Kuo *et al.*, 1995). Furthermore, *C. pneumoniae* infection has been found to establish persistent infections (Hammerschlag *et al.*, 1992; Miyashita *et al.*, 1996a; Ekman *et al.*, 1993; Falck *et al.*, 1996) and it has been reported that symptoms associated with *C. pneumoniae* infection may persist for a long time (Hammerschlag *et al.*, 1992; Miyashita *et al.*, 1996a; Ekman *et al.*, 1993; Falck *et al.*, 1996, 1997; Normann *et al.*, 1998). Clinical symptoms of *C. pneumoniae* infections are non-specific but a cough is the most common symptom and this is often prolonged (Kuo *et al.*, 1995; Hammerschlag *et al.*, 1992; Miyashita *et al.*, 1996a; Soda *et al.*, 1997). In a previous study, we encountered many cases of *C. pneumoniae* respiratory tract infection in junior high school students, and in related family members, during an outbreak of *C. pneumoniae* at their school (Soda *et al.*, 1997). During this outbreak, many students and their family members had a persistent cough that lasted for more than 2 weeks.

Patients with persistent cough frequently make multiple visits to a physician, are given several courses of antibiotics and miss time from work. If their symptoms do not improve, they may finally visit a respiratory physician. However, the role of *C. pneumoniae* infection in adult patients with persistent cough has not been well evaluated, especially in Asia. In this study, we investigated prospectively an association between persistent cough and *C. pneumoniae* infection by isolation in cell culture, by PCR from nasopharyngeal swabs and by serology.

METHODS

Study population. The patients studied were 366 adults (133 males and 233 females) between the ages of 17 and 61 years (mean age, 36·1 years). All patients had a persistent cough lasting more than 2 weeks; this time-frame has been used previously as the expected time-frame of a prolonged *C. pneumoniae* cough (Wright *et al.*, 1997). These patients were seen at the Kawasaki Medical School Hospital and Kurashiki Daiichi Hospital between April 1998 and December 2001. Patients with a cough lasting for more than 3 months, taking angiotensin-converting enzyme inhibitors or with known or suspected immunodeficiency, lung cancer, pneumonia, tuberculosis, chronic obstructive pulmonary diseases, bronchiectasis, interstitial lung diseases, chronic sinusitis, gastro-esophageal reflux, allergic rhinitis or asthma were excluded. Control subjects without complaints of a cough were selected from healthy blood donors during the study period and were matched for age, sex and smoking status. The criteria for inclusion were no signs or symptoms of acute respiratory illness during the preceding 3 months. Informed consent was obtained from all subjects.

Culture and PCR. Nasopharyngeal swab specimens were obtained from all subjects for isolation in cell culture and for PCR. Swab specimens were placed in a sucrose/phosphate/glutamate (SPG) transport medium. Each swab specimen in SPG medium was sonicated and centrifuged briefly at 900 ***g*** for 10 min. Supernatants were inoculated onto confluent monolayers of HEp-2 cells grown on round coverslips (14 mm in diameter) set in 24-well plastic cell culture plates. Plates were centrifuged at 1200 ***g*** for 60 min at room temperature. Next, 1 ml of Eagle's minimal essential medium (Nissui Pharmaceuticals) supplemented with 10 % heat-inactivated foetal calf serum (Gibco-BRL) and cycloheximide (final concentration of 1 μg ml^{-1}; Nakarai Tesque) was applied to each well. Plates were incubated in 5 % CO_2 at 35 °C for 72 h; all specimens were passaged twice (Miyashita *et al.*, 1996b, 2001c). Following incubation, a genus-specific, FITC-conjugated monoclonal antibody (mAb) (*Chlamydia* FA Seiken; Denka Seiken) and *C. pneumoniae* species-specific mAbs were used to stain inclusions (Miyashita *et al.*, 1996b). Inclusions were observed under a Nikon epifluorescence microscope at a magnification of ×200 or ×400.

C. pneumoniae-specific primers used for PCR were based on the DNA sequence within the 53 kDa protein gene established by our laboratory (Miyashita *et al.*, 1996b). This assay was performed as described previously and was carried out without prior knowledge of the culture results. The cell culture-grown *C. pneumoniae* strain KKp-15 was used as the positive control (Miyashita *et al.*, 1994). SPG transport medium was used as the negative control in every run. After electrophoresis of PCR products on a 1·5 % agarose gel at 100 V, bands were visualized by staining with ethidium bromide. The appearance of a 499 bp PCR amplification product was taken as positive. If available, follow-up nasopharyngeal swab specimens were obtained from subjects with positive findings by culture and/or PCR.

Serology. Paired serum samples were obtained from all subjects at intervals of at least 4 weeks and stored at −70 °C until testing. The microimmunofluorescence (MIF) test was used for the titration of IgG and IgM antibodies against *C. pneumoniae* (Grayston *et al.*, 1990; Kuo *et al.*, 1995) using formalin-treated elementary bodies of *C. pneumoniae* TW-183 (purchased from the Washington Research Foundation, Seattle, WA, USA) and KKpn-15 strains as antigens (Miyashita *et al.*, 1994). Sera with IgM against *C. pneumoniae* were retested after absorption with goat anti-human IgG antibody reagent (Gullsorb) to exclude false-positive reactions. An acute infection was defined as one that gave IgM titres ⩾1 : 16 or a fourfold increase in IgG or IgM titres.

Complement fixation and passive agglutinin tests were used to detect antibodies to *Mycoplasma pneumoniae*. A fourfold increase in antibody titres was taken as an indication of acute infection. Pertussis toxin (PT) and filamentous haemagglutinin (FHA) tests were performed to detect antibodies to *Bordetella pertussis* using ELISAs. Serological diagnosis of *B. pertussis* was based either on a fourfold increase in PT and/or FHA titre or a single PT or FHA result that was more than 2 SD greater than the geometric mean of the control group (Wright *et al.*, 1995).

Statistical analysis. Statistical analysis was done using the Fisher Exact test and the Chi-squared test. A mean age comparison was carried out using Student's *t*-test.

RESULTS

Study population demographics are shown in Table 1. No significant differences were observed between patients and controls with regard to age, sex or smoking status.

C. pneumoniae was detected by isolation in two patients (0·5 %) but in none of the controls ($P = 0{\cdot}902$) and by PCR in 20 patients (5·5 %) and in one control (0·9 %) ($P = 0{\cdot}048$). Both culture-positive specimens were also PCR positive. Serological evidence of acute *C. pneumoniae* infection was present in 24 patients (6·5 %) but in none of the controls ($P = 0{\cdot}004$). Thirteen cases were positive by both PCR and serology and 18 cases were positive with discrepancies in the PCR and serology results. Eleven cases were PCR negative and serology positive and seven cases were PCR positive and serology negative.

Other respiratory tract pathogens, including *B. pertussis* and *M. pneumoniae*, were identified in 68 (18·5 %) and 4 (1·1 %) patients, respectively, by serology. Dual infections of *C.*

Table 1. Study population demographics

Characteristic	Patient	Control	*P* value
Number of subjects	366	106	
Age (years; mean ± SD)	36·1 ± 10·6	36·0 ± 9·6	0·802
Age range (years)	17–61	19–60	
Sex (males; %)	133 (36·3)	38 (35·8)	0·926
Smoking status (%)			
Current	134 (36·6)	42 (39·6)	
Past	106 (29·0)	29 (27·4)	
Never	126 (34·4)	35 (33·0)	0·788*
***C. pneumoniae* infection (%)**			
Culture	2 (0·5)	0	1·000
PCR	20 (5·5)	1 (0·9)	0·048
Serology†	24 (6·5)	0	0·004

*Smokers versus 'never' smokers.

†Positive results were defined by IgM titres ⩾1 : 16 or a fourfold increase in IgG and/or IgM titres.

pneumoniae and *B. pertussis* were found in three patients but no dual infections with *M. pneumoniae* and *C. pneumoniae* or *M. pneumoniae* and *B. pertussis* were observed.

All culture-, PCR- and/or serology-positive patients with *C. pneumoniae* and *M. pneumoniae* were treated with macrolides or fluoroquinolones for 1–2 weeks. Antibiotic treatment of adult patients infected with *B. pertussis* is controversial; erythromycin is the drug of choice but, unless it is administered early, antibiotic treatment does not alter the course of the disease (Linnemann & Nasenbeny, 1977). Therefore, we treated about 30 % of our patients empirically with antibiotics. After eradication of *C. pneumoniae* from the nasopharynx, the cough disappeared in all patients. Of these patients, however, three were still positive for *C. pneumoniae* by culture and/or PCR after 2 weeks of therapy. The results of laboratory tests and treatment of these patients with persistent cough are summarized in Table 2. One patient (no. 1) was treated with clarithromycin for 2 weeks but symptoms continued and *C. pneumoniae* was still detected by both culture and PCR. After 6 weeks of therapy with clarithromycin and inhaled steroid, symptoms disappeared completely and *C. pneumoniae* was no longer detected by PCR. The other two patients (nos 2 and 3) were also treated with clarithromycin for 2 weeks and their symptoms improved. However, their PCR results for *C. pneumoniae* were still positive. At 1 or 2 weeks after antibiotic treatment was discontinued, these patients again experienced a cough and visited our outpatient clinic. They were treated with clarithromycin and inhaled steroid for 3–4 weeks. Finally, their symptoms disappeared completely and the PCR results became negative for *C. pneumoniae*.

DISCUSSION

Respiratory tract infection is one of the most common causes of persistent cough and post-infectious cough is often persistent (Poe *et al.*, 1989). Among respiratory pathogens, *B. pertussis* and *M. pneumoniae* are well-known causes of persistent cough in both children and/or adults (Wright *et al.*, 1995; Hallander *et al.*, 1999). Recently, some reports have indicated that *C. pneumoniae* infection may also cause persistent cough (Wright *et al.*, 1997; Hallander *et al.*, 1999; Kaneko *et al.*, 1999). Wright *et al.* (1997) investigated the prevalence of acute *C. pneumoniae* infection in patients presenting with persistent cough for the first time. It was found that about 20 % of patients with a cough lasting in excess of 2 weeks had serological evidence of an acute *C. pneumoniae* infection and concluded that *C. pneumoniae* infection may be responsible for a substantial proportion of prolonged coughing illnesses. However, their diagnostic methodology for *C. pneumoniae* was based on serology alone. Subsequently, Hallander *et al.* (1999) reported that *C. pneumoniae* was detected by PCR in 19 of 115 (17 %) children with coughs lasting less than 100 days. Kaneko *et al.* (1999) also detected *C. pneumoniae* by indirect immunofluorescence tests in 8 of 21 (38 %) children with persistent coughs. In contrast, Birkebæk *et al.* (2000) identified *C. pneumoniae* infection by PCR in only 3 of 201 adult patients with chronic coughs. In our study, we were able to detect *C. pneumoniae* infection by serology in 24 patients (6·5 %) and by culturing and/or PCR in 20 (5·5 %) of 366 adult patients with persistent coughs. It has been suggested that the serological criteria for the definition of an acute *C. pneumoniae* infection by the MIF test using serum is an IgG antibody titre of $\geqslant 1:512$. This is a controversial issue because of the

Table 2. Laboratory findings in three patients with persistent coughs attributed to persistent *C. pneumoniae* infection

Patient no.	Age (years)	Sex	Follow-up (days after onset of symptoms)	*C. pneumoniae* detection		Antibody titre		Duration of clarithromycin treatment (weeks)
				PCR	Culture	IgM	IgG	
1	19	M	14	+	+	128	32	2
			28	+	+	16	1024	2
			42	+	–	< 16	1024	2
			63	–	–	< 16	1024	NA
			105	–	–	< 16	512	NA
2	22	F	15	+	–	64	32	2
			36	+	–	< 16	32	3
			61	–	–	< 16	32	NA
			95	–	–	< 16	32	NA
3	27	M	25	+	–	< 16	512	2
			49	+	–	< 16	512	2
			63	+	–	< 16	512	2
			84	–	–	< 16	512	NA
			111	–	–	< 16	512	NA

NA, Not applicable.

high incidence of IgG antibodies (at titres ⩾1 : 512) seen among healthy asymptomatic subjects (Dowell *et al.*, 2001). We also made the same observation (Miyashita *et al.*, 2001c). In this study, we excluded IgG titres ⩾1 : 512 from our diagnostic criteria. If Wright *et al.* (1997) had not used the positive serological criteria of IgG titres ⩾1 : 512, 9 % (6 of 65 subjects) of subjects would have met positive serology criteria for acute *C. pneumoniae* infection by the MIF test. This prevalence rate is almost the same as our serological results. However, the prevalence in this study, as judged by PCR, was higher than that reported by Birkebæk *et al.* (2000) but much lower than that reported by Hallander *et al.* (1999). This difference may be due to the use of a different cohort from a different geographical area than that employed in their study.

The rate of infection with *M. pneumoniae* was very low among our patients and similar to other reports (Wright *et al.*, 1995; Birkebæk *et al.*, 2000). These data, together with our data, indicate that *M. pneumoniae* infection may not cause persistent cough in adults. However, several studies have demonstrated the importance of *B. pertussis* as the cause of persistent cough among adults (Wright *et al.*, 1995; Robertson *et al.*, 1987; Nennig *et al.*, 1996; Birkebæk *et al.*, 1999). These authors reported a prevalence of *B. pertussis* infection of 12–26 % in adult patients. In this study, we detected *B. pertussis* by serology in 68 patients (18·5 %) and confirmed that *B. pertussis* is a common cause of persistent cough in adults and should be considered in differential diagnosis.

C. pneumoniae is susceptible both *in vitro* and *in vivo* to macrolides, tetracyclines, ketolides and fluoroquinolones (Miyashita *et al.*, 1997, 2001a, b). However, previous reports have noted that treatment failures are common and have suggested that persistent infection may be common after a conventional course of antibiotics (Hammerschlag *et al.*, 1992; Miyashita *et al.*, 1996a, 2002; Ekman *et al.*, 1993; Falck *et al.*, 1996, 1997; Normann *et al.*, 1998; Block *et al.*, 1995; Roblin & Hammerschlag, 1998; Hammerschlag & Roblin, 2000a, b). We have also found two cases of persistent *C. pneumoniae* infection after treatment with the appropriate antibiotics against *C. pneumoniae* pneumonia (Soda *et al.*, 1997). In these two cases, we could not eradicate *C. pneumoniae* from the nasopharynx with a 2–3 week course of treatment with macrolide or tetracycline and symptoms recurred. Furthermore, we have also seen persistent *C. pneumoniae* infection for up to 2 years in patients with diffuse panbronchiolitis who were asymptomatic most of the time but who also had exacerbations (Miyashita *et al.*, 1996a).

It has been reported that there is an occurrence of continuing symptoms, relapsing illness or the development of complications with persistent infection due to *C. pneumoniae* (Hammerschlag *et al.*, 1992; Miyashita *et al.*, 1996a; Ekman *et al.*, 1993; Falck *et al.*, 1996). Anecdotal data suggest that prolonged therapy (i.e. greater than 2 weeks) may be desirable, since recrudescent symptoms have been described following erythromycin treatment for 2 weeks and even after 30 days of tetracycline or doxycycline treatment (Grayston *et al.*, 1990; Kuo *et al.*, 1995). Hammerschlag *et al.* (1992) reported refractory asthma in a patient with persistent *C. pneumoniae* infection, which was confirmed by culture, whose symptoms finally resolved after prolonged treatment with erythromycin. Furthermore, Ekman *et al.* (1993) reported that 9 of 20 patients with *C. pneumoniae* primary infection required more than one course of antibiotic therapy with erythromycin (7–14 days), doxycycline (10–14 days) or tetracycline to improve their symptoms (four patients required three courses of treatment and five patients required two courses of treatment). In contrast, Falck *et al.* (1996) reported that they were not able to eradicate *C. pneumoniae* from the nasopharynx, despite several prolonged courses of antibiotics known to be effective against *Chlamydia* species. In the present study, follow-up cultures, PCR specimens and serology results were obtained from all patients who were positive by culturing and/or PCR. In three patients, we could not eradicate *C. pneumoniae* from the nasopharynx after 2 weeks of treatment with macrolide and symptoms either continued or relapsed. Therefore, we tried prolonged macrolide therapy consisting of 5–6 weeks of administration of clarithromycin to eradicate *C. pneumoniae* or to improve symptoms. Finally, we were able to eradicate *C. pneumoniae* from the nasopharynx. When *C. pneumoniae* could no longer be detected by culture or PCR, respiratory symptoms of these patients disappeared completely and no relapse was observed. However, we were not able to assess whether eradication of *C. pneumoniae* after anti-chlamydial therapy resulted in an improvement of their cough because patients were treated simulateously with steroid inhalation. Currently, data available indicate that the optimum dose and duration of therapy for persistent *C. pneumoniae* infection are uncertain. Therefore, new treatment strategies may be necessary to eradicate the organism in patients prone to persistent infection.

In conclusion, our data indicate that *C. pneumoniae* infection may cause persistent cough in adults. Furthermore, our data also indicate that it may be necessary to eradicate the organism when *C. pneumoniae* is detected by culture and/or PCR in patients with persistent cough. More treatment studies with prolonged follow-up investigations of culture and/or PCR are needed to define more effective treatment strategies.

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