**Chlamydia pneumoniae Infection in Patients With Diffuse Panbronchiolitis and COPD**

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**Study objectives:** To determine the possible association of *Chlamydia pneumoniae* infection with diffuse panbronchiolitis (DPB) and with COPD.

**Design:** Prospective case-control study.

**Setting:** Division of Respiratory Diseases, Kawasaki Medical School Hospital.

**Participants:** Fifteen DPB and 77 COPD patients who had acute exacerbations of respiratory conditions and 35 and 120 control subjects, respectively, matched for age, sex, and smoking status.

**Measurements and results:** Nasopharyngeal swabs and paired serum samples were obtained from all patients and control subjects for isolation and antibody testing of *C pneumoniae*. *C pneumoniae* was isolated from one DPB patient and from no COPD patients or control subjects. Serologic evidence of acute *C pneumoniae* infection was observed in one DPB patient (6.7%) and six COPD patients (7.8%). The prevalence and mean titer of *C pneumoniae* IgG and IgA antibodies were significantly higher in COPD patients than in control subjects (p<0.001). However, no such differences were observed between DPB patients and control subjects.

**Conclusions:** This study showed that *C pneumoniae* infection may be associated with acute exacerbations of COPD and that chronic *C pneumoniae* infection is common in COPD but not in DPB.

(CHEST 1998; 114:969–971)

**Key words:** *Chlamydia pneumoniae*; COPD; diffuse panbronchiolitis

**Abbreviations:** DPB=diffuse panbronchiolitis; GMT=geometric mean titer

Diffuse panbronchiolitis (DPB) is a disease characterized by chronic inflammation affecting bronchioles distal to the terminal bronchiole, known as the transitional zone between airway and the pulmonary parenchyma. The obstructive respiratory functional impairment, occasional symptoms of wheezing, and cough and sputum seen in DPB patients resemble the features of COPD. However, DPB belongs to a distinctly different category from COPD because it often progresses rapidly to a fatal outcome. The onset of DPB is unrelated to age. Whether infections play a role in the clinical course and pathogenesis of DPB and COPD remains a controversial issue. Nevertheless, infection is known to exacerbate DPB and COPD, often resulting in incapacitation, respiratory failure, and even death. *Chlamydia pneumoniae* is a common cause of acute respiratory illness, including pharyngitis, bronchitis and pneumonia. Recent seroepidemiologic studies have suggested a possible association between *C pneumoniae* infection and acute exacerbations of COPD. We have seen persistent *C pneumoniae* infection for 30 months in a case of DPB with acute exacerbation. We studied the role of *C pneumoniae* in acute exacerbation of DPB and COPD by assessing the frequency of *C pneumoniae* infection with isolation and serology in patients having exacerbations of respiratory symptoms.

**Materials and Methods**

**Study Population**

Fifteen DPB patients (19 to 70 years of age, mean of 38.2 years; five men, 10 women) and 77 patients with COPD, as defined by the American Thoracic Society (51 to 81 years of age, mean of 67.0 years; 57 men, 20 women), were seen from April...
from 1993 to December 1996 at the Kawasaki Medical School Hospital for increasing dyspnea, cough, and sputum production. Patients who had received antibiotics within the 2 weeks prior to enrollment or who had an acute respiratory illness within the 2 months prior to enrollment were excluded. Pulmonary function tests (mean±SD) of DPB and COPD patients, respectively, were as follows: FEV1, 1.82±0.68 L and 1.31±0.54 L; predicted FEV1, 64.1±16.6% and 50.2±19.24%; FVC, 2.62±0.89 L and 2.56±0.78 L; and predicted FVC, 70.2±20.4% and 64.4±18.04%. Control subjects without DBP or COPD were selected from the patients attending the same hospital during the study period and matched for age, sex, and smoking status. The criteria for inclusion were no signs and symptoms of acute respiratory illness, and normal pulmonary function tests with a predicted FEV1 of 80% or greater and a predicted FVC of 80% or greater. Informed consent was obtained from all subjects.

Culture

Nasopharyngeal swab specimens were obtained for isolation from all patients and control subjects. Isolation of *C. pneumoniae* was performed by cell culture using cycloheximide-treated HEp-2 cells grown in a 24-well plate. Cultures were passed once. Cell cultures in each passage were assessed by fluorescent antibody staining using *Chlamydia* genus-specific and *C. pneumoniae* species-specific monoclonal antibodies.

Serology

Paired serum samples were collected and stored at −70°C until testing. The microimmunofluorescence test was used for titration of IgG, IgA, and IgM antibodies against chlamydial species using formalinized elementary bodies of *C. pneumoniae* KK-pn15, *Chlamydia trachomatis* L2/434/Bu, and *Chlamydia psittaci* 6BC as antigens. Rheumatoid factors were removed by absorption with Gullsorb (Gull Laboratories; Salt Lake City, Utah) before testing IgA and IgM antibodies. Acute infection was defined as IgM≥1:16, IgG≥1:512, or a fourfold rise in IgG, and chronic or preexisting antibody as IgG 1:16 to 1:256.

### Statistical Analysis

Statistical analysis was done with the two-tailed Fisher’s exact test. Mean age comparison was carried out with Student’s t test. Geometric mean titer (GMT) comparison was performed with the Wilcoxon rank sum test.

### Results

Demographic characteristics of the study population and the serologic results are shown in Table 1. No significant differences in age, sex or smoking status were observed between patients and control subjects.

*C. pneumoniae* was isolated from the nasopharynx of one DPB patient and from no COPD patients or control subjects. IgG and IgA antibodies against *C. pneumoniae* were present more often in COPD patients than in control subjects (IgG≥1:16: 96.1 vs 73.3%, *p<0.0001*; IgA≥1:16: 70.1 vs 17.5%, *p<0.0001*). The GMTs of IgG and IgA were significantly higher in COPD patients than in control subjects (IgG: 72.6 vs 19.8, *p=0.0001*; IgA: 24.0 vs 6.6, *p=0.0001*). In contrast, no significant differences in antibody frequencies (IgG≥1:16: 66.7 vs 68.6%, *p=1.000*; IgA≥1:16: 13.3 vs 17.1%, *p=1.000*) as well as GMTs (IgG: 17.5 vs 18.4, *p=0.729*; IgA: 6.1 vs 6.2, *p=0.849*) were observed between DPB patients and control subjects. However, the correlation of *C. pneumoniae*-specific IgG and IgA titer increase and age was observed in DPB patients but not in control subjects. Serologic evidence of acute *C. pneumoniae* infection was found in six COPD patients (7.8%), two of whom had IgM.

### Table 1—Demographic Characteristics and Chlamydia pneumoniae Serology

<table>
<thead>
<tr>
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<th>COPD</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Subjects</td>
</tr>
<tr>
<td>Age, yr (mean±SD)</td>
<td>67.0±6.5</td>
<td>66.8±6.2</td>
</tr>
<tr>
<td>Range</td>
<td>51-81</td>
<td>51-81</td>
</tr>
<tr>
<td>Sex, M (%)</td>
<td>57 (74.1)</td>
<td>89 (74.2)</td>
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<tr>
<td>Smoking status (%)</td>
<td>24 (31)</td>
<td>35 (29)</td>
</tr>
<tr>
<td>Current</td>
<td>40 (52)</td>
<td>61 (51)</td>
</tr>
<tr>
<td>Former</td>
<td>13 (17)</td>
<td>24 (20)</td>
</tr>
<tr>
<td>Never</td>
<td>7 (9)</td>
<td>—</td>
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<tr>
<td>Home oxygen use</td>
<td>13 (16.8)</td>
<td>—</td>
</tr>
<tr>
<td>Chronic steroid use</td>
<td>49 (63.6)</td>
<td>—</td>
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<tr>
<td>Previous hospitalization for COPD or DPB</td>
<td>7 (46.6)</td>
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<tr>
<th></th>
<th>DPB</th>
<th>Control</th>
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<tr>
<td></td>
<td>Patients</td>
<td>Subjects</td>
</tr>
<tr>
<td>C. pneumoniae antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG≥1:16 (%)</td>
<td>74 (96.1)</td>
<td>88 (73.3)</td>
</tr>
<tr>
<td>GMT</td>
<td>72.6</td>
<td>19.8</td>
</tr>
<tr>
<td>IgA≥1:16 (%)</td>
<td>54 (70.1)</td>
<td>21 (17.5)</td>
</tr>
<tr>
<td>GMT</td>
<td>24.0</td>
<td>6.6</td>
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*Ever-smokers vs never-smokers.

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antibodies, and in one DPB patient (6.7%). No acute *C. trachomatis* or *C. psittacci* infection was found. There were also no differences in frequencies of *C. trachomatis* and *C. psittacci* antibodies between DPB or COPD patients and control subjects.

**DISCUSSION**

The role of *C. pneumoniae* in exacerbations of COPD has been studied recently. In these studies, acute *C. pneumoniae* infection was detected in 4 to 5% of patients by serology. Our study showed a higher incidence (7.8%) of acute antibodies against *C. pneumoniae* in COPD patients with acute exacerbations of respiratory symptoms than was reported in two previous studies. We also found a significantly higher antibody frequency and GMTs of anti-*C. pneumoniae* IgG antibody in COPD patients with acute exacerbations of respiratory symptoms. These findings were consistent with the report by Blasi and colleagues but were contradictory to the report of Beaty and colleagues. The difference may be due to the small sample size in Beaty’s study, or periodicity of *C. pneumoniae* infection affecting the prevalence of antibody. Our data also showed an association between *C. pneumoniae*-specific serum IgA antibodies and exacerbations of respiratory symptoms in COPD. The high IgG and IgA titers observed suggested reinfection or chronic infection with *C. pneumoniae*. However, we were not able to exclude infection with other microorganisms because tests for other infections were not performed.

In contrast, the frequency and titer of *C. pneumoniae* antibody was not correlated with DPB. DPB patients were much younger than COPD patients (mean age of 38 vs 67 years), and their pulmonary function was less impaired. Therefore, it seems that DPB patients in this study were in the early stage of disease, which had not progressed to airway obstruction or respiratory symptoms as severe as those seen in COPD patients. The antibody prevalence and titers against *C. pneumoniae* were lower in DPB patients than in COPD patients, which indicates that DPB patients had been infected with *C. pneumoniae* less frequently than the COPD patients, who were much older. However, the small number of old patients with DPB who had late-stage DPB did have moderate to high IgG and IgA antibody titers. In fact, a 70-year-old man, who had had DPB for more than 20 years and was in the late or advanced stage of DPB, had maintained high antibody titers (IgG, 1:256 to 1:1024; IgA, 1:128 to 1:256) for more than 10 years. *C. pneumoniae* was isolated persistently from this patient. These findings suggest that reinfection or chronic infection with *C. pneumoniae* may be common in late or advanced stages of DPB, as in COPD. A long-term follow-up study of DPB patients may answer this question.

In conclusion, we have identified an association between *C. pneumoniae* and acute exacerbations of COPD, and shown that chronic *C. pneumoniae* infection is common in older COPD patients but not in younger DPB patients.

ACKNOWLEDGMENT. The authors thank Dr. Chou-chou Kuo, Department of Pathobiology, University of Washington, for kindly reading this manuscript and for his helpful comments.

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