Non-Specific Interstitial Pneumonia and *Chlamydia pneumoniae* Infection

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**SUMMARY:** Recently, the clinical features of non-specific interstitial pneumonia (NSIP) have been described. We hypothesize that recurrent infection caused by *Chlamydia pneumoniae* may play a role in the pathogenesis of NSIP. To prove this, we quantified serum IgA and IgG antibodies against *C. pneumoniae* using the enzyme-linked-immunosorbent assay kit. The study included 15 patients diagnosed with NSIP, 20 patients with chronic obstructive pulmonary diseases (COPD) as disease group, and 27 control subjects. IgA antibody against *C. pneumoniae* was positive in 12 of 15 patients with NSIP, in 16 of 20 patients with COPD, and in 14 of 27 control subjects. IgG antibody against *C. pneumoniae* was positive in 14 of 15 patients with NSIP, in 17 of 20 patients with COPD, and in 16 of 27 control subjects. If the cut off value (mean ± 2SD, index more than 3.0) was introduced, IgA and/or IgG antibodies against *C. pneumoniae* were positive in 8 of 15 patients with NSIP (53.3%), in 9 of 20 patients with COPD (45%), and in 2 of 27 control subjects (7.4%). These results suggest that infection of *C. pneumoniae* might play a role in the pathogenesis of NSIP.

**INTRODUCTION**

In 1994, Katzenstein and Fiorelli reported the histologic features and clinical significance of non-specific interstitial pneumonia (NSIP) (1). The histologic features of NSIP include a varying degree of interstitial inflammation and fibrosis which appear to develop over a specific time frame (i.e., the process is temporarily uniform). Recently, Park et al. reported the radiographic and CT findings (2) and clinical features (3) of seven patients with NSIP. In addition, Bjoraker et al. reported that patients with usual interstitial pneumonia (UIP) have a shorter survival than patients with other types of idiopathic chronic interstitial pneumonia including NSIP (4). More recently, Cottin et al. (5), Nagai et al. (6), and Fujita et al. (7) have described the clinical features of NSIP. However, the pathogenesis of NSIP remains unclear. Since the term "NSIP" refers to a histopathologic pattern and not a disease entity, NSIP can result from a number of causes including infections, environmental insults, and drugs (1).

*Chlamydia pneumoniae* is a frequent cause of acute respiratory infection and the most common species of *Chlamydia* in humans (8). Primary infection by *C. pneumoniae* may cause symptoms ranging from severe to mild or may even be asymptomatic (8). In addition, the role of *C. pneumoniae* in the exacerbation of chronic obstructive pulmonary diseases (COPD) has been studied (9-13). In these studies, serology revealed acute *C. pneumoniae* infection in 4 to 18% of the subjects. In addition, Miyashita et al. have reported that in 77 patients with COPD, IgA and IgG antibodies against *C. pneumoniae* are positive in 70.1% and in 96.1%, respectively (14). These findings may indicate a significant role for *C. pneumoniae* in the acute exacerbation of COPD.

McConnell et al. have described the radiographic appearance of *C. pneumoniae* (15). In Figure 3 of their article, they demonstrated a chest X-ray diagnosed as recurrent *C. pneumoniae* infection in a 68-year-old woman (15). We considered that the figure resembled a chest X-ray image of NSIP, and therefore hypothesized that chronic *C. pneumoniae* infection might be one of the causes of NSIP. With this in mind, we measured anti-*C. pneumoniae* IgA as well as IgG antibodies in patients with NSIP and patients with COPD, as a disease group, and compared the results with those of control subjects.

**PATIENTS AND METHODS**

**Patients:** Between March 1990 and November 1997, 15 patients (12 females and 3 males, median age 59; range from 39 to 70) were diagnosed with NSIP confirmed histologically (Table I). Of these, 4 had idiopathic NSIP and 11 had polymyositis (PM) or dermatomyositis (DM). PM/DM was diagnosed according to the criteria of Bohan and Peter (16): (i) symmetric muscle weakness; (ii) typical histologic findings on muscle biopsy; (iii) increased levels of muscle enzymes in the sera; (iv) compatible electromyographic findings; and (v) characteristic dermatologic manifestations.

The diagnoses of NSIP were made by open-lung biopsy. All pathologic specimens were analyzed by lung pathologists (IY and YO) according to the criteria of NSIP described by Katzenstein and Fiorelli (1). Briefly, NSIP represented a pattern of chronic interstitial pneumonia that lacked characteristic features of other specific entities such as UIP, desquamative...
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age and Sex</th>
<th>Background</th>
<th>Rheumatoid factor</th>
<th>Jo-I</th>
<th>IgA index</th>
<th>Judge</th>
<th>IgG index</th>
<th>Judge</th>
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<td>1</td>
<td>58M</td>
<td>DM</td>
<td>–</td>
<td>–</td>
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<td>4.4</td>
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<td>2</td>
<td>70F</td>
<td>DM</td>
<td>+</td>
<td>–</td>
<td>3.02</td>
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<td>2.69</td>
<td>(+)</td>
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<tr>
<td>3</td>
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<td>DM</td>
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<td>–</td>
<td>1.27</td>
<td>(+)</td>
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<td>(+)</td>
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<tr>
<td>4</td>
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<td>+</td>
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<td>(+)</td>
<td>1.95</td>
<td>(+)</td>
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<td>–</td>
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<td>(+)</td>
<td>2.43</td>
<td>(+)</td>
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<tr>
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<td>–</td>
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<td>5.98</td>
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<td>1.51</td>
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DM: dermatomyositis
PM: polymyositis

interstitial pneumonia (DIP), hypersensitivity pneumonitis, bronchiolitis obliterans organizing pneumonia (BOOP), Langerhans' cell granulomatosis, or chronic eosinophilic pneumonia. Lung biopsies in this group were characterized by varying proportions of chronic interstitial inflammation and fibrosis which were temporarily uniform.

We also evaluated 20 patients with COPD (1 female and 19 males, median age 72; range from 49 to 78) and 27 control subjects (14 females and 13 males, median age 57; range from 40 to 65). COPD was diagnosed as described previously (17).

**Blood samples:** Peripheral venous blood samples were obtained before the subjects ate breakfast. After centrifugation at 1,000 g for 10 min at 4°C, the serum was frozen and stored at -70°C until used.

**Measurement of IgA and IgG antibodies against C. pneumoniae by enzyme-linked immunosorbent assay (ELISA):** IgA and/or IgG antibodies in sera were measured by HITAZYME C. pneumoniae kit (Hitachi Chemical Co., Ltd., Tokyo) as described previously (18). The index was defined as follows:

\[
\text{Absorbance corrected by the referred serum} \div \text{Absorbance of the cut off (0.2)}
\]

An index of more than 1.0 was considered to be positive (+), and an index of more than 3.0 (mean ± 2SD of the indices of healthy persons) was considered to be strongly positive (+++) as described previously (18).

**RESULTS**

IgA antibody against C. pneumoniae was positive in 12 of 15 patients with NSIP (80%), in 16 of 20 patients with COPD (80%), and in 14 of 27 control subjects (51.9%) (Fig. 1A). IgG antibody against C. pneumoniae was positive in 14 of 15
patients with NSIP (93.3%) and in 17 of 20 patients with COPD (85%), and in 16 of 27 control subjects (59.3%) (Fig. 1B). Strongly positive IgA antibody was observed in 5 of 15 patients with NSIP (33.3%; P < 0.05 by χ-square test compared with control subjects) and in 3 of 20 patients with COPD (15%), and in 2 of 27 control subjects (7.4%) (Table 2). Strongly positive IgG antibody was observed in 4 of 15 patients with NSIP (26.7%; P < 0.05 by χ-square test compared with control subjects) and in 8 of 20 patients with COPD (40%; P < 0.01 by χ-square test compared with control subjects), and in 1 of 27 control subjects (3.7%) (Table 2). In total, strongly positive IgA and/or IgG antibodies were observed in 8 of 15 patients with NSIP (53.3%; P = 0.01 by χ-square test compared with control subjects) and in 9 of 20 patients with COPD (45%; P < 0.01 by χ-square test compared with control subjects), and in 2 of 27 control subjects (7.4%) (Table 2).

DISCUSSION

In our present study, we demonstrate that the anti-C. pneumoniae IgA and IgG antibodies determined by HITAZYME C. pneumoniae in patients with NSIP were high compared to the values of the control subjects. In addition, the levels of these antibodies were comparable with those of patients with COPD.

In the present study, IgA and/or IgG antibodies determined by HITAZYME C. pneumoniae were positive in all patients with NSIP. HITAZYME C. pneumoniae is a diagnostic reagent that has been recently developed by adopting an ELISA method for the detection of anti-C. pneumoniae antibodies (18). In addition, Kishimoto et al. have established a new diagnostic standard for HITAZYME C. pneumoniae by setting up special cut-off values for a single serum for diagnosis of C. pneumoniae infection (18). They reported that if a special cut-off value at index 3.0 for both IgA and IgG is defined (the calculation of "mean + 2SD" of healthy persons), most healthy persons fall within the range of the negative zone, and when this cut-off value is applied, the rate of strongly positive IgA and/or IgG is 7.6% for healthy persons, and 64.9% for infected patients. They determined that if index is 3.0 or greater for IgA and/or IgG, it is highly likely that the patient has an acute or a present infection (18).

In our present study, IgA and/or IgG antibodies were elevated to more than 3.0 in 8 of 15 patients with NSIP (53.3%), in 9 of 20 patients with COPD (45%), and in 2 of 27 control subjects (7.4%). Furthermore, an interesting feature of our study was that high IgA titers at presentation were found in the majority of patients with NSIP. It has been reported that elevated titers of IgA indicate reinfection with C. pneumoniae (19-23). Therefore, the high IgA and IgG titers observed in patients with NSIP suggest reinfection or chronic infection with C. pneumoniae.

Since four patients with NSIP had a rheumatoid factor, the possibility of false positive results caused by HITAZYME C. pneumoniae should be discussed. Although it has been reported that the rheumatoid factor affects the results of the anti-C. pneumoniae IgM immunofluorescence assay (24), we evaluated only IgA and IgG antibodies against C. pneumoniae. In addition, it has been reported that the rheumatoid factor does not affect these IgA and IgG ELISAs (18). Therefore, false positive results caused by the rheumatoid factor will be overlooked in our study.

The pathogenesis of C. pneumoniae infection is still largely unknown. In an animal model of C. pneumoniae infection, a mild peribronchial and perivascular lymphoid infiltration and nodular hyperplasia characterize the primary infection in the lungs (25). Reinfection results in a significant increase in the magnitude of the lymphoid reaction (25). Overall, lymphoid reactions remain detectable up to 61 days after primary challenge and rechallenge (25). This evidence may explain the increased incidence of lymphocytes in bronchoalveolar lavage fluid in patients with NSIP and its clinical course (usually subacute onset).

Our study included 11 patients with NSIP complicated with PM/DM. Therefore, the relationship between C. pneumoniae and the onset of PM/DM should be discussed. In the mouse and rabbit models of C. pneumoniae pneumonitis, the organism has been shown to spread from the respiratory tract to other organs following intranasal inoculation (26,27). In addition, it has been postulated that systemic dissemination of C. pneumoniae occurs by infection of monocytes/macrophages and consequently spreading of the organism via the blood or lymphatic circulation (28). In humans, C. pneumoniae has been detected by polymerase chain reaction in the liver, spleen, and lymph nodes during systemic disease (29). This evidence suggests that C. pneumoniae infected through the respiratory tract could spread to several organs and could cause systemic diseases. Interestingly, it has also been reported that the 60-kilodalton cysteine-rich outer membrane proteins of C. pneumoniae has sequence homology to a peptide from the marine heart muscle-specific a myosin heavy chain (30). Therefore, it can be speculated that other antigens derived from C. pneumoniae cross-react to muscle proteins and/or skin proteins, resulting in the onset of PM/DM.

Since surgical lung specimens from patients with NSIP are available, future studies to detect C. pneumoniae antigen by immunohistochemistry, or to detect C. pneumoniae DNA using in situ hybridization or polymerase chain reaction, should be performed.

In summary, we describe the first evidence that IgA and/or IgG anti-C. pneumoniae antibodies were elevated in patients with NSIP. Recurrent C. pneumoniae infection might play a role in the pathogenesis of NSIP.

REFERENCES