Jpn. J. Infect. Dis., 54, 225-228, 2001

Original Article

Non-Specific Interstitial Pneumonia and *Chlamydia pneumoniae* Infection

Jiro Fujita*, Shuji Bandoh, Michiaki Tokuda, Yuji Ohtsuki[1], Ichiro Yamadori[2], Takeo Yoshinouchi[3] and Toshihiko Ishida

First Department of Internal Medicine, Kagawa Medical University, Miki-cho 1750-1, Kita-gun, Kagawa 761-0793,
[1]Department of Pathology, Kochi Medical School, Kochi 783-8505,
[2]Department of Pathology, National Okayama Medical Center, Okayama 700-8558 and
[3]Department of Internal Medicine, Nagoya City University, Nagoya 467-8602, Japan

(Received August 14, 2001. Accepted November 6, 2001)

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 1). 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

*Corresponding author: Tel: +81-87-891-2145, Fax: +81-87-891-2147, E-mail: jiro@kms.ac.jp

Table 1. Patient characteristics

Case	Age and Sex	Background	Rheumatoid factor	Jo-1	IgA index	Judge	IgG index	Judge
1	58M	DM	–	–	<0.9	(–)	4.4	(++)
2	70F	DM	+	–	3.02	(++)	2.69	(+)
3	39F	DM	–	–	1.27	(+)	1.77	(+)
4	65F	PM	–	–	1.62	(+)	1.95	(+)
5	56F	PM	–	+	2.55	(+)	2.43	(+)
6	62F	PM	–	–	2.33	(+)	2.16	(+)
7	58M	PM	–	–	<0.9	(–)	1.77	(+)
8	55F	DM	–	+	<0.9	(–)	2.3	(+)
9	59F	PM	+	–	2.09	(+)	5.98	(++)
10	57F	DM	–	+	3.49	(++)	3.34	(++)
11	66M	DM	–	–	4.14	(++)	0.91	(–)
12	67F	idiopathic	+	–	2.75	(+)	3.49	(++)
13	70F	idiopathic	–	–	1.84	(+)	1.99	(+)
14	53F	idiopathic	–	–	3.73	(++)	1.8	(+)
15	69F	idiopathic	+	–	5	(++)	1.51	(+)

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:

$$\frac{\text{Absorbance corrected by the referred serum}}{\text{Absorbance of the cut off (0.2)}}$$

An index of more than 1.0 was considered to be positive (+), and an index of more than 3.0 (mean ± 2SD of the indexes 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

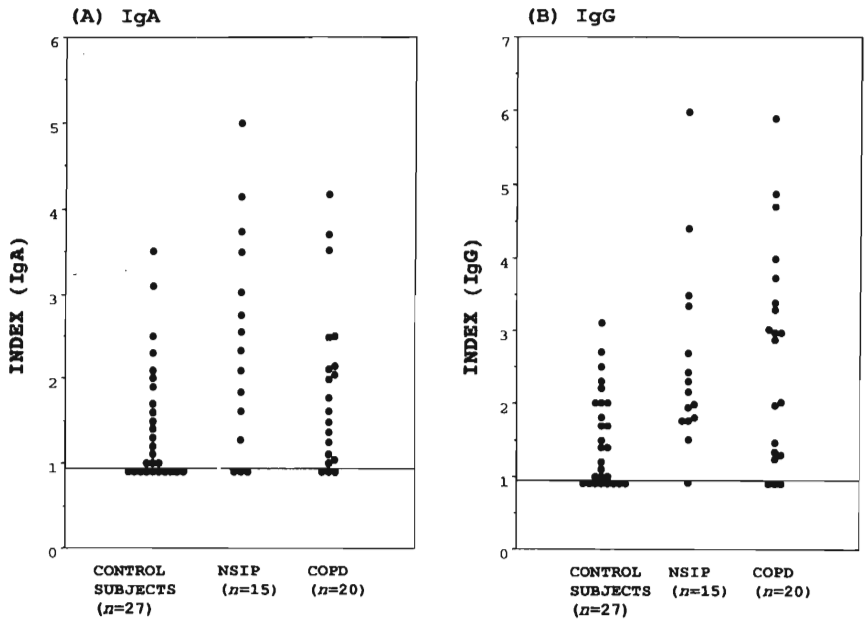

Fig. 1. Titers of anti-*Chlamydia pneumoniae* IgA (A) and IgG (B) antibodies in patients with NSIP, COPD, and control subjects. An index of more than 1.0 is considered to be positive (+), and an index of more than 3.0 is considered strongly positive (++).

Table 2. Strongly positive rates of IgA antibody, IgG antibody, and IgA and/or IgG antibodies against *Chlamydia pneumoniae* in NSIP, COPD, and control subjects

	IgA	IgG	IgA and/or IgG
NSIP	5/15 (33%)*	4/15 (27%)*	8/15 (53%)***
COPD	3/20 (15%)	8/20 (40%)**	9/20 (45%)**
Control	2/27 (7%)	1/27 (4%)	2/27 (7%)

NSIP: non-specific interstitial pneumonia
COPD: chronic obstructive pulmonary disease
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control

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%; $P < 0.001$ 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 > index 3.0 for either IgA 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 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 increase 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 murine 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

1. Katzenstein, A. and Fiorelli, R. F. (1994): Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. Am. J. Surg. Pathol., 18, 136-147.
2. Park, J. S., Lee, K. S., Kim, J. S., Park, C. S., Suh, Y. L., Choi, D. L. and Kim, K. J. (1995): Nonspecific interstitial pneumonia with fibrosis: radiographic and CT findings in seven patients. Radiology, 195, 645-648.
3. Park, C. S., Jeon, J. W., Park, S. W., Lim, G. I., Jeong, S. H., Uh, S. T., Park, J. S, Choi, D. L., Jin, S. Y. and Kang, C. H. (1996): Nonspecific interstitial pneumonia/fibrosis:

clinical manifestations, histologic and radiologic features. Korean J. Intern. Med., 11, 122-132.
4. Bjoraker, J. A., Ryu, J. H., Edwin, M. K., Myers, J. L., Tazelaar, H. D., Schroeder, D. R. and Offord, K. P. (1998): Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med., 157, 199-203.
5. Cottin, V., Donsbeck, A. V., Revel, D., Loire, R. and Cordier, J. F. (1998): Nonspecific interstitial pneumonia. Individualization of a clinicopathologic entity in a series of 12 patients . Am. J. Respir. Crit. Care Med., 158, 1286-1293.
6. Nagai, S., Kitaichi, M., Itoh, H., Nishimura, K., Izumi, T. and Colby, T. V. (1998): Idiopathic nonspecific interstitial pneumonia/fibrosis: comparison with idiopathic pulmonary fibrosis and BOOP. Eur. Respir. J., 12, 1010-1019.
7. Fujita, J., Yamadori, I., Suemitsu, I., Yoshinouchi, T., Ohtsuki, Y., Yamaji, Y., Kamei, T., Kobayashi, M., Nakamura, Y. and Takahara, J. (1999): Clinical features of non-specific interstitial pneumonia. Respir. Med., 93, 113-118.
8. Kuo, C. C., Jackson, L. A., Campbell, L. A. and Grayston, J. T. (1995): *Chlamydia pneumoniae* (TWAR). Clin. Microbiol. Rev., 8, 451-461.
9. Beaty, C. D., Grayston, J. T., Wang, S. P., Kuo, C. C., Reto, C. S. and Martin, T. R. (1991): *Chlamydia pneumoniae*, strain TWAR, infection in patients with chronic obstructive pulmonary disease. Am. Rev. Respir. Dis., 144, 1408-1410.
10. Blasi, F., Legnani, D., Lombardo, V. M., Negretto, G. G., Magliano, E., Pozzoli, R., Chiodo, F., Fasoli, A. and Allegra, L. (1993): *Chlamydia pneumoniae* infection in acute exacerbations of COPD. Eur. Respir. J., 6, 19-22.
11. Von Hertzen, L., Alakarppa, H., Koskinen, R., Liippo, K., Surcel, H. M., Leinonen, M. and Saikku, P. (1997): *Chlamydia pneumoniae* infection in patients with chronic obstructive pulmonary disease. Epidemiol. Infect., 118, 155-164.
12. Soler, N., Torres, A., Ewig, S., Gonzalez, J., Celis, R., El-Ebiary, M., Hernandez, C. and Rodriguex-Roisin, R. (1998): Bronchial microbial patterns in severe exacerbations of chronic obstructive pulmonary disease (COPD) requiring mechanical ventilation. Am. J. Respir. Crit. Care Med., 157, 1498-1505.
13. Mogulkoc, N., Karakurt, S., Isalska, B., Bayindir, U., Celikel, T., Korten, V. and Colpan, N. (1999): Acute purulent exacerbation of chronic obstructive pulmonary disease and *Chlamydia pneumoniae* infection. Am. J. Respir. Crit. Care Med., 160, 349-353.
14. Miyashita, N., Niki, Y., Nakajima, M., Kawane, H. and Matsushima, T. (1998): *Chlamydia pneumoniae* infection in patients with diffuse panbronchiolitis and COPD. Chest, 114, 969-971.
15. McConnell, C. T. Jr., Plouffe, J. F., File, T. M., Mueller, C. F., Wong, K. H., Skelton, S. K., Marson, B. J. and Breiman, R. F. (1994): Radiographic appearance of *Chlamydia pneumoniae* (TWAR strain) respiratory infections. CBPIS Study Group. Community-based Pneumonia Incidence Study. Radiology, 192, 819-824.
16. Bohan, A. and Peter, J. B. (1975): Polymyositis and dermatomyositis (First of two parts). New Engl. J. Med., 292, 344-347.
17. American Thoracic Society. (1995): Standards for the diagnosis and care of patients with COPD. Am. J. Respir. Crit. Care Med., 157, 1498-1505.
18. Kishimoto, T., Matsushima, T., Morikawa, T. and Kawagoe, K. (1999): Assay of specific anti-*Chlamydia pneumoniae* antibodies by ELISA method. 3. Setting of serological criteria. J. Jpn. Assoc. Infect. Dis., 73, 457-466 (in Japanese).
19. Saikku, P., Leinonen, M., Tenkanen, L., Linnanmaki, E., Ekman, M. R., Manninen, V., Manttari, M., Frick, M. H. and Huttunen, J. K. (1992): Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. Ann. Intern. Med., 116, 273-278.
20. Eknan, M. R., Grayston, J. T., Visakorpi, R., Kleemola, M., Kuo, C. C. and Saikku, P. (1993): An epidemic of infections due to *Chlamydia pneumoniae* in military conscripts. Clin. Infect. Dis., 17, 420-425.
21. Hahn, D. L., Dodge, R. and Golubjatnikov, R. (1991): Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis and adult-onset asthma. JAMA, 266, 225-230.
22. von Hertzen, L., Leinonen, M., Surcel, H. M., Karjalainen, J. and Saikku, P. (1995): Measurement of sputum antibodies in the diagnosis of acute and chronic respiratory infections associated with *Chlamydia pneumoniae*. Clin. Diagn. Lab. Immunol., 2, 454-457.
23. Hahn, D. L., Anttila, T. and Saikku, P. (1996): Association of *Chlamydia pneumoniae* IgA antibodies with recently symptomatic asthma. Epidemiol. Infect., 117, 513-517.
24. Verkooyen, R. P., Hazenberg, M. A., Van Haaren, G. H., Van Den Bosch, J. M., Snijder, R. J., Van Helden, H. P. and Verbrugh, H. A. (1992): Age-related interference with *Chlamydia pneumoniae* microimmunofluorescence serology due to circulating rheumatoid factor. J. Clin. Microbiol., 30, 1287-1290.
25. Penttila, J. M., Anttila, M., Puolakkainen, M., Laurila, A., Varkila, K., Sarvas, M., Makela, P. H. and Rautonen, N. (1998): Local immune responses to *Chlamydia pneumoniae* in the lungs of BALB/C mice during primary infection and reinfection. Infect. Immun., 66, 5113-5118.
26. Yang, Z. P., Kuo, C. C. and Grayston, J. T. (1995): Systemic dissemination of *Chlamydia pneumoniae* following intranasal inoculation in mice. J. Infect. Dis., 171, 736-738.
27. Moazed, T. C., Kuo, C., Patton, D. L., Grayston, J. T. and Campbell, L. A. (1996): Experimental rabbit models of *Chlamydia pneumoniae* infection. Am. J. Pathol., 148, 667-676.
28. Moazed, T. C., Kuo, C. C., Grayston, J. T. and Campbell, L. A. (1998): Evidence of systemic dissemination of *Chlamydia pneumoniae* via macrophages in the mouse. J. Infect. Dis., 177, 1322-1325.
29. Grayston, J. T. (1994): *Chlamydia pneumoniae* (TWAR) infections in children. Pediatr. Infect. Dis. J., 13, 675-685.
30. Bachrnaier, K., Neu, N., de la Maza, L. M., Pal, S., Hessel, A. and Penninger, J. M. (1999): Chlamydia infections and heart disease linked through antigenic mimicry. Science, 283, 1335-1339.