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CONCISE REPORT

Acute *Chlamydia pneumoniae* infection in the pathogenesis of autoimmune diseases

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Autoimmune diseases have several etiologies. Acute *Chlamydia pneumoniae* (*C. pneumoniae*) infection may be involved in the pathogenesis of several autoimmune diseases. In this study, 82 patients with several autoimmune diseases and 70 controls were enrolled, and acute *C. pneumoniae* infection has been evaluated by monitoring the levels of IgM antibody. *Chlamydia pneumoniae* IgM positive results were observed in 29% ($P < 0.05$) of the patients with several autoimmune diseases and in 10% of the controls. *Chlamydia pneumoniae* IgM positive cases were more frequent among the patients with rheumatoid arthritis (RA; 30%, $P < 0.05$), systemic lupus erythematosus (SLE; 28.0%, $P < 0.05$), dermatomyositis/polymyositis (23%, NS), myeloperoxidase-antineutrophil cytoplasmic autoantibody (MPO-ANCA)-associated vasculitis (33%, NS), adult onset of Still's disease (29%, NS) and giant cell arteritis/Takayasu arteritis (50%, NS) than among the controls. This positive frequency was statistically significant in RA and SLE. These results suggest that acute *C. pneumoniae* infection is probably involved in the pathogenesis of autoimmune diseases. *Lupus* (2009) **18,** 164–168.

Key words: autoimmune diseases; *C. pneumoniae*

Introduction

Autoimmune diseases have several etiologies. Some authors have suggested that infection by several microorganisms such as parvovirus,[1] HTLV-1,[2] *Mycoplasma fermentans*,[3] *Mycoplasma pneumoniae (M. pneumoniae)*,[4] Epstein-Barr virus[5] and cytomegalovirus[5,6] could be involved in the pathogenesis of autoimmune diseases. However, the relationship between *Chlamydia pneumoniae* (*C. pneumoniae*) infection and autoimmune diseases has not been thoroughly evaluated yet. Only few studies showed that *C. pneumoniae* infection could be involved in the pathogenesis of myeloperoxidase-antineutrophil cytoplasmic autoantibody (MPO-ANCA)-associated glomerulonephritis,[7,8] reactive arthritis (ReA),[9] systemic lupus erythematosus (SLE)[10] and adult onset of Still's disease (AOSD).[11] In this study, the relationship between C. *pneumoniae* infection and several autoimmune diseases including rheumatoid arthritis (RA), SLE, dermatomyositis/polymyositis (DM/PM), MPO-ANCA-associated vasculitis, AOSD and giant cell arteritis/Takayasu arteritis (GCA/TA) was further evaluated by monitoring the levels of *C. pneumoniae* IgM antibody.

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Patients and methods

Patients

We enrolled in this study patients with RA ($n = 27$), SLE ($n = 25$), DM/PM ($n = 13$), MPO-ANCA-associated vasculitis ($n = 6$), AOSD ($n = 7$) and GCA/TA ($n = 4$). All cases fulfilled the revised criteria of the American College of Rheumatology. Evaluation for *C. pneumoniae* IgM titres was performed in the acute phase of the underlying diseases before treatment with steroids and/or disease-modifying antirheumatic drugs.

Exclusion criteria

It is known that *C. pneumoniae* IgM antibody starts to increase after 2 weeks and its high titres persist at least

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for 7 weeks. Therefore, in the present study, we excluded cases diagnosed as having autoimmune diseases more than 9 weeks before our evaluation for the *C. pneumoniae* IgM titres. Also, cases with compromised health status such as those receiving chemotherapy and/or immunosuppressive therapy were excluded.

Controls

Seventy controls were selected from patients with preoperative admission, osteoarthritis and healthy volunteers. The absence of history of autoimmune diseases was confirmed by checking their medical records.

Serology

An ELISA kit (Hitazyme *C. pneumoniae*) was used to measure the levels of *C. pneumoniae* IgM antibody as a maker of acute *C. pneumoniae* infection.[12] The ELISA kit detects antibody to the chlamydial outer membrane complex excluding lipopolysaccharide, which is isolated from the purified elementary bodies of the *C. pneumoniae* YK-41 strain.[13] The concentration of *C. pneumoniae* IgM antibody in each sample was expressed as IgM index. Index value >1.60 was considered positive for IgM antibody as previously described.[14]

Statistical analysis

Statistical analyses were performed using Fisher's exact *t*-test with SPSS statistical program. All *P* values were two-sided, and $P < 0.05$ was considered as statistically significant.

Results

The distributions of *C. pneumoniae* IgM antibody index in the autoimmune cases ($n = 82$) and the controls ($n = 70$) are shown in Figure 1. *Chlamydia pneumoniae* IgM antibody index in the autoimmune cases was apparently higher than that in the controls. *Chlamydia pneumoniae* IgM positive results were observed in 29% ($P < 0.05$) of the patients with several autoimmune diseases and in 10% of the controls (Table 1). *Chlamydia pneumoniae* IgM positive cases were significantly more frequent among the patients with RA (30%, $P < 0.05$) and SLE (28%, $P < 0.05$) than among the controls (10%) (Table 1). In addition, *C. pneumoniae* IgM positive cases were more frequent among the patients with DM/PM (23%, NS), ANCA-associated vasculitis (33%, NS), AOSD (29%, NS) and GCA/TA (50%, NS) than among the controls (10%) (Table 1), but the differences were not statistically significant because the examined cases were few. No significant relationship was found between the presence of *C. pneumoniae* IgM antibody and organ involvement in each autoimmune disease (data not shown).

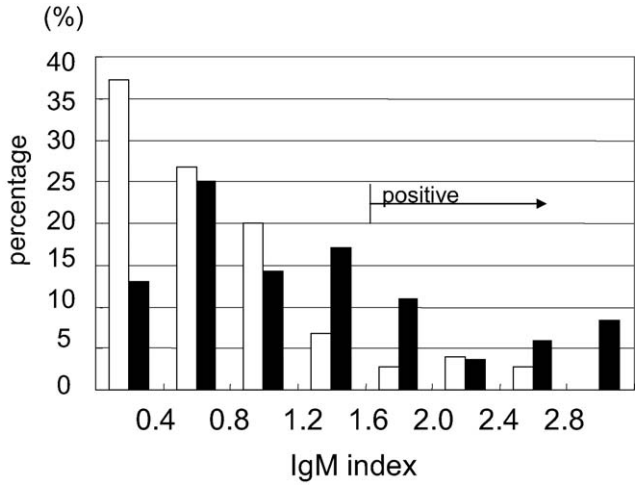

Figure 1 The distribution of *Chlamydia pneumoniae* IgM antibody index in the autoimmune cases (black) ($n = 82$) and controls (white) ($n = 70$). The *C. pneumoniae* IgM antibody index in the autoimmune cases was apparently higher than that in the controls.

It is generally known that *C. pneumoniae* causes an upper respiratory tract infection.[15] Therefore, we investigated whether a preceding infection with clinical manifestations can be detected or not. However, a preceding symptomatic upper respiratory infection could be detected only in seven cases (one with RA, three with SLE, one with DM, one with AOSD and one with ANCA-vasculitis) of 24 autoimmune cases with elevated *C. pneumoniae* IgM titres (data not shown). The other 17 cases were asymptomatic.

Table 1 The prevalence of *Chlamydia pneumoniae* IgM antibodies in autoimmune diseases

Diseases	*No. of patients*	*No. of positive (%)*[a]	P *Value*
RA	27	8 (30%)	<0.05
SLE	25	7 (28%)	<0.05
DM/PM	13	3 (23%)	NS
ANCA-vasculitis	6	2 (33%)	NS
AOSD	7	2 (29%)	NS
GCA/TA	4	2 (50%)	NS
Total	82	24 (29%)	<0.05
Control	70	7 (10%)	

Abbreviations: RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; DM/PM, dermatomyositis/polymyositis; ANCA, ; AOSD, adult onset of Still's disease; GCA/TA, giant cell arteritis/Takayasu arteritis.
[a]Index value >1.60 is defined as positive for IgM antibodies.

Also, we evaluated the temporal relationship between the onset of autoimmune diseases and the *C. pneumoniae* infection. The *C. pneumoniae* IgM antibody was detected after the onset in all 24 cases (three cases: after 0–2 weeks, seven cases: among 2–4 weeks, five cases: among 4–6 weeks, five cases: among 6–8 weeks, four cases: among 8–9 weeks). Surprisingly, the *C. pneumoniae* IgM antibody was not detected before the onset even in any of the seven upper respiratory symptomatic patients. Also in those cases, the *C. pneumoniae* IgM antibody was detected after the onset (two cases: after 0–2 weeks, two cases: after 2–4 weeks, two cases: after 4–6 weeks, one case: after 6–8 weeks). No significant difference in the detection period of the *C. pneumoniae* IgM antibody was found between cases having upper respiratory symptoms and those without symptoms.

Discussion

The relationship between autoimmune diseases and the increase of C. pneumoniae *IgM titres*

We showed herein that *C. pneumoniae* IgM positive cases were more frequent among the patients with several autoimmune diseases than among the controls. This positive frequency was statistically significant in RA and SLE. Moreover, *C. pneumoniae* IgM positive cases were more frequent among the patients with DM/PM, ANCA-associated vasculitis, AOSD and GCA/TA than among the controls, but the differences were not statistically significant because the examined cases in our study were few. A large-scaled study may provide more reliable results in the future.

Previously, Iyoda, *et al.*[8] found increases of the *C. pneumoniae* IgM titres in 9 (60%) of 15 active MPO-ANCA-associated glomerulonephritis patients and in 13 (26%) of 50 controls. Braun, *et al.*[9] noticed increases of the *C. pneumoniae* IgM titres in 5 (7%) of 70 patients with either ReA ($n = 11$) or undifferentiated oligoarthritis ($n = 59$) and in 0 (0%) of 58 controls. In these reports, based on the increases of the *C. pneumoniae* IgM titres, the authors suggested that autoimmune diseases probably develop after acute infection with *C. pneumoniae*,[8,9] as in our cases. Thus, our results suggest that acute *C. pneumoniae* infection could be involved in the pathogenesis of autoimmune diseases.

It is generally known that *C. pneumoniae* causes an upper respiratory tract infection. However, a preceding symptomatic upper respiratory infection could be detected only in 7 of 24 autoimmune cases with increases of *C. pneumoniae* IgM titres. This suggests that the infection may be asymptomatic in most cases. Alternatively, this may result from inadequate investigations for the *C. pneumoniae* infection in the patients with autoimmune diseases. Braun, *et al.*[9] described five patients with ReA associated with *C. pneumoniae*. Three of these patients had a preceding symptomatic upper respiratory tract infection, whereas the other two patients had not. Because these five patients showed *C. pneumoniae* specific cellular immune response and humoral immune response, the investigators suggested that ReA can develop after an upper respiratory *C. pneumoniae* infection irrespective of the presence of symptomatic manifestations. Their findings are in accordance with ours.

The hypothesis that acute *C. pneumoniae* infection may be involved in the pathogenesis of autoimmune diseases is based on increases of the *C. pneumoniae* IgM titres. Therefore, we further evaluated the association between the *C. pneumoniae* infection and the onset of autoimmune diseases. First, to exclude the possibility of simply reflecting the serum IgM elevation, the ratio of the *C. pneumoniae* IgM titres (index) against the total amount of serum IgM (mg/dL) was calculated. This ratio in the *C. pneumoniae* IgM positive cases was apparently higher than in the negative cases (mean values: 0.019 vs 0.0084, respectively). This means that the increases of *C. pneumoniae* IgM titres were specific. Second, it is known that *C. pneumoniae* IgM antibody increases only after primary *C. pneumoniae* infection. However, the *C. pneumoniae* IgA and IgG antibodies increase after primary and secondary *C. pneumoniae* infections. Therefore, increases of the *C. pneumoniae* IgM titres only mean primary *C. pneumoniae* infection. Third, we evaluated the temporal relationship between the onset of autoimmune diseases and acute *C. pneumoniae* infection. In no case was the *C. pneumoniae* IgM antibody detected before the disease onset even in seven upper respiratory symptomatic patients. In general, the *C. pneumoniae* IgM antibody starts to increase 2 weeks after the infection and reaches a plateau after 4–5 weeks, and its high titres persist for 2 weeks. Thereafter, the IgM antibody gradually declines and disappears 9–10 weeks after the infection. Thus, detection of *C. pneumoniae* IgM antibody is often behind for several weeks. However, if the *C. pneumoniae* IgM antibody could be detected within 2 weeks after the onset, it is likely that the *C. pneumoniae* infection precedes the onset at least in those cases. In this regard, of 24 cases with positive *C. pneumoniae* IgM, three were positive within 2 weeks after the onset. We could conclude that *C. pneumoniae* infection developed before the disease onset in those three cases. Regarding the seven respiratory symptomatic

patients, the respiratory symptoms preceded the onset of autoimmune diseases by 2–10 days. It is likely that the *C. pneumoniae* infection preceded the onset in those cases. However, even in those cases, the *C. pneumoniae* IgM antibody was not detected before the onset. It is difficult to accurately determine whether the *C. pneumoniae* infection precedes the onset in other cases, judging from the date of positive results of the *C. pneumoniae* IgM antibody.

Therefore, we also examined the possibility of the *C. pneumoniae* infection after the onset. In this respect, we have to consider the possibility that the presence of autoimmune diseases might increase the risk for *C. pneumoniae* infection due to immuno-dysregulation by the diseases themselves or pre-existing conditions as background of the diseases. In that case, the *C. pneumoniae* infection may occur without any effects on the development of the diseases. First, if the presence of autoimmune diseases increases the risk for infection after the onset, they could also increase the risk for infection by other agents except *C. pneumoniae*. Therefore, we investigated whether infection with *M. pneumoniae* and *Streptococcus pyogenes* (*S. pyogenes*) could be detected or not. The *M. pneumoniae* IgM antibody was detected in only 2 of 24 autoimmune cases with *C. pneumoniae* infection. Similarly, the *S. pyogenes* antigen (pharynx) was detected in only one of those cases (data not shown). Second, we checked for the presence of immuno-suppression and evaluated the difference in the general immunological parameters such as white blood cell (WBC) counts (including neutrophils and lymphocytes) and immunoglobulin level (total IgM and IgG titres) between *C. pneumoniae* IgM positive and negative patients. There were no significant differences in the cell counts between both groups (*C. pneumoniae* IgM positive patients versus negative patients; WBC counts: 7200/μL vs 8300/μL, neutrophils: 5000/μL vs 6400/μL, lymphocytes: 1400/μL vs 1400/μL). The immunoglobulin levels in the *C. pneumoniae* IgM positive patients were slightly higher than that in the negative patients (IgM: 150 mg/dL vs 120 mg/dL and IgG: 1900 mg/dL vs 1500 mg/dL). Therefore, it is not likely that the presence of these autoimmune diseases increases the risk for infection in the examined cases. Also, these findings lower the possibility that the *C. pneumoniae* infection developed after the onset of autoimmune diseases. In other words, it increases the possibility that the *C. pneumoniae* infection precedes the onset of autoimmune diseases.

The reason why *C. pneumoniae* infection causes systemic diseases is still unclear. One explanation is molecular mimicry.[16] Immune responses and/or antibodies against unknown *C. pneumoniae* antigen might cross-react with several body tissues such as the kidney, blood vessels, skin, joint and/or muscle. Alternatively, *C. pneumoniae* can survive and replicate within the epithelial cells, macrophages and neutrophils.[17] *Chlamydia pneumoniae*-infected cells may then trigger abnormal signal transduction, resulting in changes of the cytokine profiles and activation and/or deactivation of immunocytes such as B cells, T cells, macrophages and natural killer (NK) cells.[18,19] *Chlamydia pneumoniae* is known to produce heat shock protein (HSP)60,[15] which is involved in the initial defense against infection by introducing γδT cells. Therefore, it is possible that the γδT cells of patients with autoimmune diseases may respond to HSP60 of *C. pneumoniae* and produce various cytokines.

In conclusion, *C. pneumoniae* IgM positive results were observed in several autoimmune diseases. Statistically significant results were noticed in RA and SLE. Our results indicate that acute *C. pneumoniae* infection may be involved in the pathogenesis of autoimmune diseases. However, the exact pathogenic role of the *C. pneumoniae* infection in the development of autoimmune diseases remains unclear and needs further investigations.

Conflicts of interest

The authors have declared none.

References

1 White, DG, Woolf, AD, Mortimer, PP, *et al.* Human parvovirus arthropathy. *Lancet* 1985; **1**: 419–421.
2 Iwakura, Y, Tosu, M, Yoshida, E, *et al.* Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-1. *Science* 1991; **253**: 1026–1028.
3 Gilroy, CB, Keat, A, Robinson, DT. The prevalence of Mycoplasma fermentans in patients with inflammatory arthritides. *Rheumatology* 2001; **40**: 1355–1358.
4 Ramirez, AS, Rosas, A, Beriain, JA, *et al.* Relationship between rheumatoid arthritis and Mycoplasma pneumoniae: a case-control study. *Rheumatology* 2005; **44**: 912–914.
5 Billings, PB, Hoch, SO, White, PJ, *et al.* Antibodies to the Epstein-Barr virus nuclear antigen and to rheumatoid arthritis nuclear antigen identify the same polypeptide. *Proc Natl Acad Sci U S A* 1983; **80**: 7104–7108.
6 Barzilai, O, Sherer, Y, Ram, M, *et al.* Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report. *Ann NY Acad Sci* 2007; **1108**: 567–577.
7 Iyoda, M, Hato, T, Matsumoto, K, *et al.* Rapidly progressive glomerulonephritis in a patient with Chlamydia pneumoniae infection: a possibility of superantigenic mechanism of its pathogenesis. *Clin Nephrol* 2006; **65**: 48–52.
8 Iyoda, M, Kuroki, A, Sugisaki, T. Chlamydia pneumoniae infection and MPO-ANCA–associated glomerulonephritis. *Nephrol Dial Transplant* 2007; **22**: 965–966.
9 Braun, J, Laitko, S, Treharne, J, *et al.* Chlamydia pneumoniae- a new causative agent of reactive arthritis and undifferentiated oligoarthritis. *Ann Rheum Dis* 1994; **53**: 100–105.

10 Kitumnuaypong, T, Scalzi, LV, Nalbant, S, *et al*. Is there a role for Chlamydia pneumoniae infection in systemic lupus erythematosus and in the associated atherosclerotic cardiovascular disease. *Clin Exp Rheum* 2004; **22**: 339–342.

11 Takeda, H, Ling, M, Ochi, M, *et al*. A patient with adult Still's disease with an inceased Chlamydia pneumoniae antibody titer. *J Infect Chemother* 2002; **8**: 262–265.

12 Kishimoto, T, Kubota, Y, Matsushima, T, *et al*. Assay of specific anti-Chlamydia pneumoniae antibodies by ELISA method. 1. Evaluation of ELISA kit using outer membrane complex. *Kansenshogaku Zasshi* 1996; **70**: 821–829.

13 Miyashita, N, Kanamoto, Y, Matsumoto, A. The morphology of Chlamydia pneumoniae. *J Med Microbiol* 1993; **38**: 418–425.

14 Miyashita, N, Obase, Y, Fukuda, M, *et al*. Evaluation of serological tests detecting Chlamydophila pneumoniae-specific immunogloblin M antibody. *Inter Med* 2006; **45**: 1127–1131.

15 Kuo, CC, Jackson, LA, Campbell, LA, *et al*. Chlamydia pneumoniae (TWAR). *Clin Micro Rev* 1995; **8**: 451–461.

16 Blank, M, Barzilai, O, Shoenfeld, Y. Molecular mimicry and autoimmunity. *Clin Rev Allergy Immunol* 2007; **32**: 111–118.

17 Varsat, AD, Balana, ME, Wyplosz, B. Chlamydia-host cell Interactions: recent advances on bacterial entry and intracellular development. *Traffic* 2004; **5**: 561–570.

18 Kotwal, GJ. Microorganisms and their interaction with the immune system. *J Leukoc Biol* 1997; **62**: 415–429.

19 Von Herrath, MG, Fujinami, RS, Whitton, JL. Microorganisms and autoimmunity: making the barren field fertile. *Nat Rev Microbiol* 2003; **1**: 151–157.