Association of Seropositivity for Antibody to Chlamydia-Specific Lipopolysaccharide and Coronary Artery Disease in Japanese Men

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Recent studies suggest an association between Chlamydia pneumoniae infection and coronary artery disease (CAD). To examine this relationship in Japanese men, serum IgA and IgG antibodies to Chlamydia-specific lipopolysaccharide were measured by enzyme-linked immunosorbent assay in 507 patients with CAD and 200 age-matched controls. CAD patients were divided into (1) 269 patients with myocardial infarction (MI) and (2) 238 patients with chronic coronary heart disease (CCHD). Compared with the control group, the CAD group did not differ in the prevalences of both antibodies (IgA: 23.7 vs 18.0%, p=0.10; IgG: 52.7 vs 51.0%, p=0.6). The index of IgG antibody was not significantly different between CAD and control groups (median 1.19 vs 1.18, p=0.3), whereas the index of IgA antibody was significantly higher in CAD than control group (median 0.60 vs 0.46, p<0.0001). Compared with the control group, the MI group had a significantly higher prevalence of IgA antibody (28.6 vs 18.0%, p=0.007); however, there was no difference in the prevalence of IgG antibody (58.0 vs 51.0%, p=0.13). The CCHD group did not differ in the prevalences of both antibodies (IgA: 18.1 vs 18.0%, p=0.9; IgG: 45.6 vs 51.0%, p=0.2). After the adjustment for coronary risk factors, odds ratios (ORs) of seropositive antibodies for CAD were 1.59 [95% confidence interval (CI): 0.88–2.87, p=0.12] for IgA seropositivity and 0.92 (95% CI: 0.58–1.47, p=0.7) for IgG seropositivity in all cases. In the MI and control groups, ORs of seropositive antibodies for MI were 2.67 (95% CI: 1.32–5.38, p=0.007) for IgA seropositivity, and 1.36 (95% CI: 0.79–2.36, p=0.2) for IgG seropositivity. This study discovered that IgA antibody to Chlamydia was significantly associated with CAD, especially with MI, in Japanese Men and the findings suggest that chronic infection of Chlamydia may be linked to the pathogenesis of MI.  (Jpn Circ J 2001; 65: 182–187)

Key Words: Chlamydia pneumoniae; Chlamydia lipopolysaccharide; Coronary artery disease; Myocardial infarction

Increased attention is being given to the association between inflammation and atherosclerosis. Among the several biological factors that participate in inflammatory processes, infection is considered as one of the most important. Recent studies have shown a relationship between atherosclerosis and infectious agents such as Herpes simplex, Cytomegalovirus, Helicobacter pylori and Chlamydia pneumoniae. C. pneumoniae is a newly isolated species of Chlamydia and causes infection of the upper respiratory tract, pneumonia, bronchitis, pharyngitis and sinusitis. Pathological studies have demonstrated the presence of C. pneumoniae in coronary atherosclerotic lesions and epidemiological studies based on measuring the antibodies to Chlamydia have also shown significantly higher titers of antibodies to C. pneumoniae, measured by the microimmunofluorescence (MIF) test or enzyme-linked immunosorbent assay (ELISA), in patients with coronary artery disease (CAD). Most of the previous studies investigating this association have been performed in North America and Europe with a few small investigations conducted in the Japanese population. To assess the association of chlamydial infection with CAD in Japanese men, we therefore conducted a cross-sectional study, consisting of 707 subjects, by measuring the levels of IgA and IgG antibodies to Chlamydia-specific lipopolysaccharide (LPS) by ELISA.

Methods

Study Population
This study comprised 707 Japanese men, consisting of 507 patients (mean age, 60.0 years) with CAD defined as more than 50% stenosis in at least one major coronary artery and 200 healthy subjects (mean age, 60.8 years). We measured 1,245 samples from coronary angiography undertaken at Juntendo University between April 1996 and December 1999 and excluded 193 samples from females, 451 samples that were duplicated, and 94 samples that had less 50% stenosis in the major coronary artery. Therefore, a total of 507 patients with CAD were enrolled. The CAD group was divided into 269 patients with myocardial infarct-
tion (MI) and 238 patients with chronic coronary heart disease (CCHD), according to the clinical history, electrocardiogram (ECG) and left ventriculogram. The MI group consisted of 130 acute MI (AMI) and 139 old MI patients. MI was defined as the presence of typical prolonged chest pain lasting for 30 min associated with elevation of creatine kinase above twice the upper limit of normality and the appearance of abnormal Q waves and/or inversion of T waves on the ECG. CCHD was defined as either angina pectoris or ECG changes of more than 0.1 mV ST-depression during the exercise stress test with normal ventriculograms. We were able to measure 385 healthy subjects who visited 2 medical centers in Tokyo for their annual check-up between February and May 1998. After excluding female samples and selecting by age range, a total 200 age-matched healthy men were enrolled for the control group. None of them had a history of angina or MI and all had normal ECG examinations. All subjects gave informed consent and the local ethical committee approved this study.

Measurement of the Levels of Antibodies to Chlamydia-Specific LPS

Serum samples for measurements of antibodies to Chlamydia and lipid profiles were obtained from the patient groups just before the CAG examination after overnight fasting, and from the control group in the morning after overnight fasting. In patients who had emergency CAG, serum samples for measurements of antibodies to Chlamydia were obtained at the beginning of CAG, and serum samples for lipid profiles were obtained after overnight fasting. The levels of IgA and IgG antibodies to Chlamydia-specific LPS were measured by ELISA kits (Medac, Hamburg, Germany), as described previously31–33 which are based on a chemically pure recombinant LPS containing a genus-specific epitope of Chlamydia spp.33 The lower limit of a chemically pure recombinant LPS containing a genus-specific epitope of Chlamydia spp.33

The prevalence of antibodies to Chlamydia-LPS and CAD was compared by the chi-square test, quantitative values, except for the indices of antibodies to Chlamydia, were analyzed by Student’s t test, and the indices of antibodies to Chlamydia were compared by Mann-Whitney’s U test. The independent association between the prevalence of seropositive Chlamydia-specific antibodies and the patient groups was evaluated by multiple logistic regression analysis. A p value less than 0.05 was considered statistically significant.

Results

Clinical Characteristics and Lipid Profiles

Table 1 shows the clinical characteristics of the study populations. Body mass index (BMI) was significantly higher in the CAD group than in the control group (p=0.03). Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were significantly lower in the CAD group than in the control group (p<0.0001). The prevalences of hypertension, diabetes, and smoking history were significantly higher in the CAD group than in the control group (p<0.0001). Furthermore, the clinical characteristics in the control, MI, and CCHD groups were compared. There was no difference in age among the 3 groups. Compared with the control group, the prevalences of hypertension, diabetes, and smoking history were significantly higher (p<0.0001), and the values of TC and HDL-C were significantly lower (p<0.0001), in the MI and CCHD groups. Comparing the MI and the CCHD groups, these values and prevalences were not different (Table 1).

Prevalence of Seropositivity and Indices for Chlamydia-Specific LPS

Table 2 shows the prevalence of seropositivity and median indices for Chlamydia-specific LPS IgA and IgG antibodies. Compared with the control group, the CAD group showed no significant prevalence of seropositivity for IgA (23.7 vs 18.0%, p=0.10) or IgG (52.7 vs 51.0%, p=0.6). The index of IgG antibody was not significantly different between the CAD and control groups (median 1.19 vs 1.18, p=0.3), whereas the index of IgA antibody was significantly higher in the CAD group than in the control group (0.60 vs 0.46, p<0.0001).

Fig 1 is a comparison of the indices of Chlamydia-specific LPS antibodies between the control and patient groups with single-vessel disease or multi-vessel disease. Compared with the control group, there was no difference for IgG antibodies in patients with single-vessel (1.06 vs 1.18, p=0.9) or multi-vessel disease (1.20 vs 1.18, p=0.2), whereas there were significantly higher indices for IgA antibodies in patients with single-vessel disease (0.62 vs 0.46, p=0.005) and in

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<th>Table 1 Characteristics of the Study Groups</th>
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<td><strong>Control</strong> (n=200)</td>
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Data are mean ± SD. BMI, body mass index; HDL, high-density lipoprotein; CAD, coronary artery disease; MI, myocardial infarction; CCHD, chronic coronary heart disease.
patients with multi-vessel disease (0.60 vs 0.46, p<0.0001). There was no difference between the indices of both antibodies in the patients with single-vessel or multi-vessel disease. Furthermore, the indices of both antibodies in the MI group were analyzed, but there was no difference between the patients with single-vessel or multi-vessel disease.

Fig 2 shows the prevalences of seropositivity for IgA and IgG antibodies for Chlamydia-specific LPS in the MI, CCHD and control groups. There was no difference between the prevalences of seropositivity for both antibodies in the CCHD and the control groups (IgA: 18.1 vs 18.0%, p=0.9; IgG: 45.6 vs 51.0%, p=0.2). Compared with the control group, the MI group had a significantly higher prevalence of IgA antibody (28.6 vs 18.0%, p=0.007); however, there was no difference for IgG antibody (58.0 vs 51.0%, p=0.13). Compared with the CCHD group, the MI group had significantly higher prevalences of seropositivity for IgA antibody (28.6 vs 18.1%, p=0.0006) and IgG antibody (58.0 vs 45.6%, p=0.005).

Odds Ratios for CAD, CCHD, and MI
Table 3 shows the odds ratios (ORs) for CAD compared with the control group based on seropositivity for antibodies to Chlamydia-specific LPS, before and after adjustment for age, BMI, hypertension, diabetes, smoking, TC and HDL-C. The ORs of seropositive antibodies for CAD were 1.41 (95% confidence interval (CI): 0.93–2.14, p=0.10) for IgA antibody and 1.07 (95% CI: 0.77–1.48, p=0.6) for IgG antibody. After adjusting for confounding factors, ORs were 1.59 (95% CI: 0.88–2.87, p=0.12) for IgA antibody and 0.92 (95% CI: 0.58–1.47, p=0.7) for IgG antibody. ORs of sero-positive antibodies for CCHD were 1.00 (95% CI: 0.60–1.63, p=0.9) for IgA antibody and 0.80 (95% CI: 0.55–1.18, p=0.2) for IgG antibody, and after adjusting for confounding factors, ORs were 0.93 (95% CI: 0.45–1.93, p=0.8) for IgA antibody and 0.70 (95% CI: 0.40–1.22, p=0.2) for IgG antibody.

Table 3 also shows the ORs for MI compared with the control group based on seropositivity for antibodies to Chlamydia-specific LPS, before and after adjustment for coronary risk factors. The ORs of seropositive antibodies for MI were 1.83 (95% CI: 1.17–2.85, p=0.007) for IgA antibody and 1.33 (95% CI: 0.92–1.92, p=0.13) for IgG antibody. After adjusting for confounding factors, the ORs of
seropositive antibodies for MI were 2.67 (95% CI: 1.32–5.38, p=0.006) for IgA antibody and 1.36 (95% CI: 0.79–2.36, p=0.2) for IgG antibody.

Discussion

The present study had 3 main results.

(1) There was a significant association between the index of IgA antibody to Chlamydia-specific LPS and CAD in Japanese men.

(2) The index of IgA antibody in patients with single-vessel disease and with multi-vessel disease was higher than in control subjects; however, there was no difference between the patients with single-vessel or multi-vessel disease.

(3) After dividing the CAD group into MI and CCHD groups, and adjusting for confounding factors, there was significant association between seropositivity for IgA antibody and MI; however, there was no significant difference between the CCHD and control groups.

We have previously reported that patients with CAD tended to have a high prevalence of seropositivity for antibodies to Chlamydia-LPS and in small sized study patients with acute coronary syndrome (ACS) had a significantly higher prevalence of seropositivity for antibodies to Chlamydia than either controls or CAD patients without prior ACS. The present cross-sectional study of Japanese men further supports these findings.

The present results indicate an association of chlamydial infection with the pathophysiology of MI. C. pneumoniae is known to chronically infect macrophages, endothelium and vascular smooth muscle cells. Recent reports have shown that macrophages infected by C. pneumoniae induced cytokine production, including tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and chlamydial heat shock protein 60 has been localized in the macrophages of human atherosclerotic plaque where it induced production of TNF-α, IL-6, and matrix metalloproteinase. Vulnerable plaque prone to rupture is the cause of MI and one of the most important factors regulating the vulnerability of atherosclerotic plaque might be inflammation of the shoulder of the plaque by infection with Chlamydia. Recent studies have reported an association between Chlamydia infection and vascular thrombosis so Chlamydia infection might not only predispose the plaque to rupture, but also participate in the subsequent occlusion of vessels. A recent clinical study and 2 interventional trials of treatment with macrolide antibiotics support the hypothesis that there is a causative association between chlamydial infection and MI.

After adjustments for confounding and traditional coronary risk factors, such as age, smoking, hypertension, diabetes, TC and HDL-C, seropositivity for IgA antibody to Chlamydia was independently related to MI; however, we did not find a relationship with seropositivity for IgG antibody. It has been reported that IgA antibody is catabolized 5–6 times faster than IgG antibody so seropositivity for IgA antibody to Chlamydia is thought to reflect chronic Chlamydia infection. The present study further supports the results of recent prospective study that showed an association between IgA antibodies to C. pneumoniae and subsequent risk of death from ischemic heart disease.

Several investigations showed a lack of association between seropositivity for antibodies to C. pneumoniae and acute or chronic CAD. Ridker et al did not find a significant association of seropositivity for antibody to C. pneumoniae and MI in the Physician’s Health Study. However, that prospective study measured only IgG antibody at baseline, but not IgA antibody. The study by Altman et al also only measured IgG antibody to C. pneumoniae and Celli et al studied subjects without differentiating AMI and other patient groups.

The present study showed that there were no differences in the indices for antibodies to Chlamydia between the patients with single-vessel disease and those with multi-vessel disease. It is possible that Chlamydia infection is associated with the pathogenesis MI rather than with the severity of the diseased vessels. AMI does not necessarily involve patients with multi-vessel disease because the rupture of the vulnerable plaque mainly occurs in mild lesions of coronary stenosis, based on angiographic diagnosis.

It was recently reported that coronary angioplasty induced a rise in C. pneumoniae antibodies; however, there was no significant difference in the antibodies to Chlamydia-LPS before and after elective coronary stenting in consecutive 136 patients of another study (data not shown). The prevalence of seropositivity for both antibodies was not significantly different between the AMI and old MI groups in the present study (IgA: AMI 26.2% vs old MI 30.9%; IgG: AMI 56.2% vs old MI 59.7%). If the hypothesis that Chlamydia infection might be one of the causal factors in the pathogenesis of AMI is correct, the titers of antibodies to Chlamydia could change during the clinical course of AMI. Our next step is to investigate the changes in the antibodies to Chlamydia during the course of AMI.

Study Limitations

This study could not demonstrate a direct association between Chlamydia infection and MI, because of the nature of a cross-sectional study. We measured antibodies to Chlamydia spp, not specifically C. pneumoniae; however,
the elevated serum levels of antibody to Chlamydia are generally related in most patients to infection with C. pneumoniae. It has been reported that MIF and other ELISA methods used in previous studies can cross-react with other species of Chlamydia; therefore, these methods are not necessarily the ‘golden standard’ in measuring the antibody to C. pneumoniae. Furthermore, a recent study in patients with AMI showed a higher prevalence of seropositivity for chlamydial antibody measured by the ELISA used in our study than by the MIF method; so that method might reflect a different activity in chlamydial infection compared with the MIF test.

**Conclusion**

This study indicated that IgA antibody to Chlamydia was significantly associated with CAD, especially with MI, in Japanese Men. Re-infection and reactivation of chronic, persistent chlamydial infection might cause plaque disruption and/or thrombotic events in a proportion of patients with MI. Further well-designed prospective interventional trials are required to confirm theses findings.

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