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# Serologic and Histopathologic Study of *Chlamydia pneumoniae* Infection in Atherosclerosis: A Possible Pathogenetic Mechanism of Atherosclerosis Induced by *Chlamydia pneumoniae*

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## Abstract

Chronic infection and inflammation have recently been implicated as important etiologic agents for atherosclerosis in general and, in particular, ischemic heart disease. Several agents have been suggested as possible candidates for the chronic inflammation including *cytomegalovirus, Helicobacter pylori* and *Chlamydia pneumoniae*. We hypothesized that a vascular infection with *C. pneumoniae* may induce a chronic inflammatory reaction in the host vascular tissue and activated inflammatory cells may express inflammatory mediators such as cyclooxygenase-2 (COX-2) and matrix metalloproteinases (MMPs). At first, we evaluated the relationship between *C. pneumoniae* infection and atherosclerosis indirectly by serologic study, and then, to confirm our hypothesis, we performed an immunohistochemical study of atherosclerotic plaques. The seropositive rate of anti-*Chlamydia pneumoniae* IgG was higher in the disease group (Group I, 59.8%, n=254) than in the negative control group (Group III, 47.4%, n=97) (p=0.041), but the anti-*Chlamydia pneumoniae* IgA was not different in seropositivity between the two groups (Group I, 64.6%; Group III, 57.7%). The simultaneous seropositive rates of both IgG and IgA were 56.7% in Group I and 43.3% in Group III (p=0.033). In subgroups without the conventional risk factors of atherosclerosis, these findings were more prominent. Furthermore, we performed immunohistochemical staining on the atherosclerotic aortic tissues obtained from patients that were seropositive to *C. pneumoniae* (n=5), by using antibodies to *C. pneumoniae*, COX-2, and MMP-9. The immunoreactivity for COX-2 and MMP-9 increased in the atherosclerotic plaques itself, predominantly in the surrounding area of immunoreactive *C. pneumoniae*. These findings support our hypothesis and *C. pneumoniae* may participate in a pathogenetic mechanism for atherogenesis or progression of atherosclerosis. The present study may open a promising perspective concerning future therapeutic trials of chronic inflammation related atherogenesis under pathophysiological conditions.

Key Words: *Chlamydia pneumoniae*, atherosclerosis, cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMPs)

## INTRODUCTION

Atherosclerosis is currently considered to be an exaggerated response of the vessel wall to injury characterized by inflammation and fibrocellular proliferation rather than a degenerative disease owing to hemodynamic loading.[1,2] This view is supported by the demonstration of abundant macrophages and T lymphocytes in atherosclerotic plaque, and studies examining markers of inflammation (e.g., C-reactive protein).[3-5] Plaque rupture leading to thrombosis is the key event in myocardial infarction and has been shown to be related to increased inflammation within the plaque, rather than plaque morphology or the degree of vessel stenosis.[6] Chronic inflammatory lesions are often associated with the significant destruction of connective tissue. The association of the monocyte/macrophage with lesions in which there is a destruction of connective tissue in atherosclerotic tissue favors their participation in the breakdown of collagen.[7]

In addition, chronic infection and inflammation

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have recently been implicated as important etiologic agents for atherosclerosis in general and ischemic heart disease in particular. Several agents have been suggested as being responsible for chronic inflammation including *Cytomegalovirus, Helicobacter pylori,* and *Chlamydia pneumoniae.*[8-10] Chlamydiae spp. are Gram-negative obligatory intracellular bacteria responsible for a wide range of chronic, progressive inflammatory conditions. *C. pneumoniae*, one of the four Chlamydial species, commonly causes a spectrum of lower and upper respiratory tract diseases in humans.[11] The first association between *C. pneumoniae* and atherosclerotic diseases was reported in Finland,[12] in which high titers of IgG and IgA antibodies aginst *C. pneumoniae* occurred significantly more often in men with myocardial infarction and in those with chronic coronary heart disease than in age matched and randomly selected controls. Thereafter, although many studies have found an association,[13-15] other studies have found no independent association. This suggests that other compounding factors such as socioeconomic status or pathologically innocent bystand may be involved. It is unclear whether a possible link between *C. pneumoniae* infection and atherosclerosis would be by direct infection of the atherosclerotic plaque or by so far unexplained systemic effect.

In this study, our goal was to confirm the relationship between *C. pneumoniae* and atherosclerosis indirectly by seroepidemiologic study and then to determine the possible pathogenetic role of *C. pneumoniae* infection on atherosclerosis by histopathologic study.

## MATERIALS AND METHODS

### Seroepidemiologic study

Among patients with typical symptoms of angina and with positive results in non-invasive testing (EKG, Treadmill test) from May 1997 to Sep. 1998, 391 patients who underwent coronary angiogram were included in this study. Among them, the patients who demonstrated luminal narrowing of more than 50% in at least one vessel were grouped into the disease group (Group I, n=254) and those patients who had normal coronary arteries or minimal lesion were grouped into the positive control group (Group II, n=137). We also studied healthy persons who had not experienced any symptoms related to coronary heart disease and had normal findings on noninvasive tests for coronary artery disease and these subjects were grouped into the negative conrtol group (Group III, n=97). Serologic tests for anti-Chlamydial IgG and IgA were performed using ELISA kit (Bioclone Inc., Sydney, Australia). We obtained the mean OD value from duplicate tests and the cut-off value of the Sample Index (SI) calculated from the OD value was 1.1.

### Tissue preparation and Histologic examination

The study population consisted of five patients (four men and one woman; range of age, 51−74 years) with atherosclerotic aortic aneurysm (two patients) and dissection (three patients) of aorta, who were referred to Severance Hospital (Seoul, Korea) for evaluation and surgical treatment between 1990 and 1998. All patients were seropositive to *C. pneumoniae* either with IgG or IgA antibodies. For the control studies, specimens of normal aorta were obtained from two patients (ages, 20 and 26 years) who were surgically treated for traumatic aortic dissection.

Immediately following the removal of the aortic segments, each segment was fixed with buffered 10% formalin in order to maintain vascular morphologic integrity. To preserve the integrity of the adventitia and perivascular tissues, aortic specimens were carefully removed in a segment along with adjacent tissues and rinsed with PBS (phosphate buffered saline). Each segment was embedded in paraffin and cut in 5 m sections, which were then stained with hematoxylin-eosin (H & E). Sections of these tissues were also used for the immunohistochemical staining procedures.

### Immunohistochemistry for *Chlamydia pneumoniae*, COX-2, MMP-9 and TIMP-1

Mouse anti-*Chlamydia pneumoniae* monoclonal antibody (RR-402) (DAKO Inc., Carpenteria, CA, USA) and goat polyclonal antibodies against human COX-2, MMP-9 and TIMP-1 (tissue inhibitor of metalloproteinase) (Santa-Cruz Biotechnology Inc., Santa Cruz, CA, USA) were used as the primary antibodies for immunohistochemical staining. To characterize the type of infected cells, HAM56 (monoclonal mouse anti-human macrophage) was used for the immunostaining of the tissue type of macrophages/mononu-

clear cells. Anti-Chlamydial monoclonal antibody reacts with a major outer membrane protein (MOMP) of *C. pneumoniae* and the immunogen was *C. pneumoniae* strain TW183. Peroxidase-conjugated secondary antibodies were used with these primary antibodies.

The paraffin sections were deparaffinized and rehydrated and then the sections were boiled with citric acid for 5 minutes in order to suppress nonspecific binding of the antibodies and to increase the exposure of antigens, and cooled at room temperature for 20 minutes. The sections were then treated with 0.3% $H_2O_2$ for 5 minutes to suppress endogenous peroxidase activity. After treatment with PBS (pH=7.2-7.4) for 5 minutes and application of 1 : 5 diluted anti-Chlamydial primary antibodies (RR-402) and 1 : 100 diluted COX-2, MMP-9, TIMP-1, and HAM56 primary antibodies, the sections were incubated in a moist chamber for 1 hour. After washing and bathing for 5 minutes by PBS, the biotinylated secondary antisera cocktail including goat anti-mouse and anti-rabbit IgG diluted 1/400 was incubated on the slides for 15 minutes at room temperature in a moist chamber. The sections were then processed by the streptavidin-biotin-peroxidase complex method by use of the LSAB (+) kit (DAKO Inc., Carpenteria, CA, USA) and DAB solution (Research Genetics Inc., Huntsville, AL, USA) in order to produce a brown color at the site of reactivity. The sections were then couterstained with Mayer's hematoxyline.

### Statistical analysis

We used SPSSWIN 8.0 software for the statistical

**Table 1. Demographic Characteristics of the Disease and Control Groups**

| Risk factors | Group I | Group II | Group III | p-value |
|---|---|---|---|---|
| Age (Year) | 60.3±10.4 | 56.7±9.5 | 48.2±12.1 | 0.000 |
| Male | 160 (63.0%) | 69 (50.4%) | 55 (56.7%) | 0.051 |
| Female | 94 (37.0%) | 68 (49.6%) | 42 (43.3%) | |
| Smoker | 118 (46.5%) | 50 (36.5%) | 46 (47.4%) | 0.122 |
| Nonsmoker | 136 (53.5%) | 87 (63.5%) | 51 (52.6%) | |
| Hypertension | 132 (52.0%) | 60 (43.8%) | 15 (15.5%) | 0.000 |
| Normotensive | 122 (48.0%) | 77 (56.2%) | 82 (84.5%) | |
| Diabetes | 70 (27.6%) | 12 (8.8%) | 10 (10.3%) | 0.000 |
| Non-diabetes | 184 (72.4%) | 125 (91.2%) | 87 (89.7%) | |
| T.Chol (mg/dl)* | 195.4±45.1 | 190.9±42.1 | 201.1±34.7 | 0.239 |
| HDL-Chol (mg/dl)* | 42.8±23.9 | 42.2±20.8 | 48.6±12.8 | 0.056 |
| LDL-Chol (mg/dl)* | 118.7±34.9 | 119.0±39.8 | 123.6±34.8 | 0.547 |

*Mean±SD.

**Table 2. Seropositive Rate of IgG and IgA Antibodies against *Chlamydia pneumoniae***

| | Group I | Group II | Group III | p-value | | | |
|---|---|---|---|---|---|---|---|
| | | | | Group I vs II | Group I vs III | Group II vs III | Group I+II vs III |
| IgG | 59.8% | 67.2% | 47.4% | NS | 0.041 | 0.004 | 0.010 |
| IgA | 64.6% | 74.5% | 57.7% | NS | NS | 0.011 | NS |
| IgG & IgA | 56.7% | 61.3% | 43.3% | NS | 0.033 | 0.010 | 0.011 |

$\chi^2$ test; NS, not significant ($p>0.05$).

**Table 3. Seropositive Rates in Group I and III, Subgrouped by Known Risk Factors of Coronary Heart Disease**

| | Risk factors | Seropositivity (%) | | p-value | OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|---|---|---|
| | | Group I | Group III | | | |
| IgG | Age (Year) ≥55 | 65.6 | 53.3 | NS | | |
| | <55 | 58.1 | 55.2 | NS | | |
| | Male | 62.5 | 60.0 | NS | | |
| | Female | 55.3 | 31.0 | 0.015 | 2.8 (1.3−6.0) | 6.1 (1.6−23.0)* |
| | Smoker | 61.9 | 56.5 | NS | | |
| | Nonsmoker | 58.1 | 39.2 | 0.032 | 2.2 (1.1−4.1) | 3.7 (1.3−10.8)† |
| | Hypertension | 55.3 | 40.0 | NS | | |
| | Normotensive | 64.8 | 48.8 | 0.034 | 1.9 (1.1−3.4) | |
| | Diabetes | 50.7 | 70.0 | NS | | |
| | Non-diabetes | 63.1 | 44.8 | 0.007 | 2.1 (1.3−3.5) | |
| | T.Chol (mg/dl) ≥240 | 45.0 | 46.7 | NS | | |
| | <240 | 63.3 | 47.6 | 0.025 | 1.9 (1.1−3.2) | |
| | HDL-Chol (mg/dl) <35 | 62.5 | 50.0 | NS | | |
| | ≥35 | 61.9 | 46.6 | 0.047 | 1.9 (1.0−3.3) | |
| | LDL-Chol (mg/dl) ≥160 | 63.2 | 50.0 | NS | | |
| | <160 | 61.9 | 46.9 | 0.044 | 1.8 (1.1−3.2) | |
| IgA | Age (Year) ≥55 | 69.8 | 76.7 | NS | | |
| | <55 | 51.6 | 50.7 | NS | | |
| | Male | 63.8 | 58.2 | NS | | |
| | Female | 66.0 | 57.1 | NS | | |
| | Smoker | 66.1 | 60.9 | NS | | |
| | Nonsmoker | 63.2 | 54.9 | NS | | |
| | Hypertension | 62.1 | 86.7 | NS | | |
| | Normotensive | 67.2 | 52.4 | 0.048 | 1.9 (1.0−3.3) | |
| | Diabetes | 70.1 | 60.0 | NS | | |
| | Non-diabetes | 62.6 | 57.5 | NS | | |
| | T.Chol (mg/dl) ≥240 | 45.0 | 60.0 | NS | | |
| | <240 | 63.3 | 57.3 | NS | | |
| | HDL-Chol (mg/dl) <35 | 60.7 | 50.0 | NS | | |
| | ≥35 | 61.0 | 58.0 | NS | | |
| | LDL-Chol (mg/dl) ≥160 | 57.9 | 50.0 | NS | | |
| | <160 | 61.2 | 59.3 | NS | | |
| IgG & IgA | Age (Year) ≥55 | 62.5 | 53.3 | NS | | |
| | <55 | 38.7 | 38.8 | NS | | |
| | Male | 59.4 | 52.7 | NS | | |
| | Female | 52.1 | 31.0 | 0.035 | 2.4 (1.1−5.2) | 5.2 (1.4−18.6)‡ |
| | Smoker | 59.3 | 56.5 | NS | | |
| | Nonsmoker | 54.4 | 31.4 | 0.008 | 2.6 (1.3−5.2) | 3.9 (1.3−11.0)§ |
| | Hypertension | 52.3 | 46.7 | NS | | |
| | Normotensive | 61.5 | 42.7 | 0.013 | 2.1 (1.2−3.8) | |
| | Diabetes | 47.8 | 70.0 | NS | | |
| | Non-diabetes | 59.9 | 40.2 | 0.004 | 2.2 (1.3−3.7) | |
| | T.Chol (mg/dl) ≥240 | 35.0 | 53.3 | NS | | |
| | <240 | 58.6 | 41.5 | 0.016 | 2.0 (1.2−3.4) | |
| | HDL-Chol (mg/dl) <35 | 57.6 | 55.6 | NS | | |
| | ≥35 | 58.1 | 42.0 | 0.038 | 1.9 (1.1−3.4) | |
| | LDL-Chol (mg/dl) ≥160 | 52.6 | 56.3 | NS | | |
| | <160 | 58.3 | 40.7 | 0.018 | 2.0 (1.2−3.5) | |

NS, not significant; OR, odds ratio.
* p=0.007, † p=0.016, ‡ p=0.012, § p=0.012.

analysis and the seropositive rate of each group was compared by *Chi*-square test for univariate analysis and logistic regression for multivariate analysis. $P < 0.05$ was regarded as a statistically significant difference.

## RESULTS

### Seroepidemiologic study

**Demographic characteristics of patients and control groups:** A total 488 persons were included in this seroepidemiologic study for anti-*Chlamydia pneumoniae* IgG and IgA (254 in the disease group, 137 in the positive control group, and 97 in the negative control group). Their demographic characteristics were as shown in Table 1.

**IgG:** The seropositive rates of anti-*Chlamydia pneumoniae* IgG were 59.8%, 67.2%, and 47.4% in Group I, II, and III, respectively, and there was a significant difference noted between Group I and III $\chi^2$ test, p=0.041) and the odds ratio (OR) was 1.66 (95% CI; 1.07−2.58). Upon subgrouping by conventional risk factors, the seropositive rates in Group I and Group III were 55.3% and 31.0% (p=0.015, OR =2.8) in females, 58.1% and 39.2% (p=0.032, OR =2.2) in non-smokers, 64.8% and 48.8% (p=0.034, OR=1.9) in patients with normal blood pressure, 63.1% and 44.8% (p=0.007, OR=2.1) in non-diabetes, 63.3% and 47.6% (p=0.025, OR=1.9) in patients with normal cholesterol level, 61.9% and 46.6 % (p=0.047, OR=1.9) in patients with high HDL-cholesterol level (35 mg/dl), and 61.9% and 46.9% (p=0.044, OR=1.8) in patients with low LDL-cholesterol level (<160 mg/dl), respectively. By multivariate analysis using Logistic regression, a statistically significance was noticed in the female (p=0.007, OR =6.1 (95% CI; 1.6−23.0)) and non-smoker (p= 0.016, OR=3.7 (95% CI; 1.3−10.8)) subgroups (Table 2 and 3).

**IgA:** The seropositive rates of anti-*Chlamydia pneumoniae* IgA were 64.6%, 74.5%, and 57.7% in Group I, II, and III, respectively, and there was no significant difference between Group I and III. In subgrouping by conventional risk factors, the seropositive rates in Group I and Group III were different only in the subgroup with normal blood pressure (67.2% and 52.4%, p=0.048, OR=1.9). However, a statistical difference was not noticed in any subgroups by multivariate analysis (Table 2 and 3).

**Both IgG and IgA:** The simultaneous seropositive rates of both IgG and IgA were 56.7%, 61.3%, and 43.3% in Group I, II, and III, respectively, and there was a significant difference between Group I and III (p=0.033, OR=1.71 (95% CI; 1.07−2.75)). In subgrouping by conventional risk factors, the seropositive rates of both IgG and IgA in Group I and Group III, respectively, were 52.1% and 31.0% (p=0.035, OR=2.4) in females, 54.4% and 31.4% (p=0.008, OR=2.6) in non-smokers, 61.5% and 42.7% (p= 0.013, OR=2.1) in patients with normal blood pressure, 59.9% and 40.2% (p=0.004, OR=2.2) in non-diabetes, 58.6% and 41.5% (p=0.016, OR=2.2) in patients with normal cholesterol level (<240 mg/dl), 58.1% and 42.0% (p=0.038, OR=1.9) in patients with high HDL-cholesterol level (≥35 mg/dl), and 58.3% and 40.7% (p=0.018, OR=2.0) in patients with low LDL-cholesterol level (<160 mg/dl). By multivariate analysis using Logistic regression, a statistical significance was noticed in females (p=0.012, OR=5.2 (95% CI; 1.4−18.6)) and non-smokers (p= 0.012, OR=3.9 (95% CI; 1.3−11.0) (Table 2 and 3).

### Histopathologic analysis

The sections of aorta taken from traumatic dissections showed no histological evidence of atherosclerosis, except for minimal intimal thickening, and showed normal patterns of elastic media. In contrast to the control group, the five case-specimens showed a thickened intima from necrosis and a lipid-laden plaque formation that characterized the diseased aortas. There was also a prominent inflammatory infiltration with mononuclear cells and foam cells in the atherosclerotic plaque.

*C. pneumoniae* was stained (dark brown color) within the plaque macrophages/ mononuclear cells in four out of five diseased aortas and neither of the two control aortas. The control aortic tissues revealed little immunoreactivity for COX-2 and MMP-9 in the minimal thickened intima, and no immunoreactivity in the media. In the atherosclerotic lesions, however, the immunoreactivity for COX-2 and MMP-9 was evident in all cases of aortic atherosclerosis along with plaques, primarily in macrophages/foam cells, intimal and medial smooth muscle cells, and endothelial cells of the intima. Within the intima, the increased im-

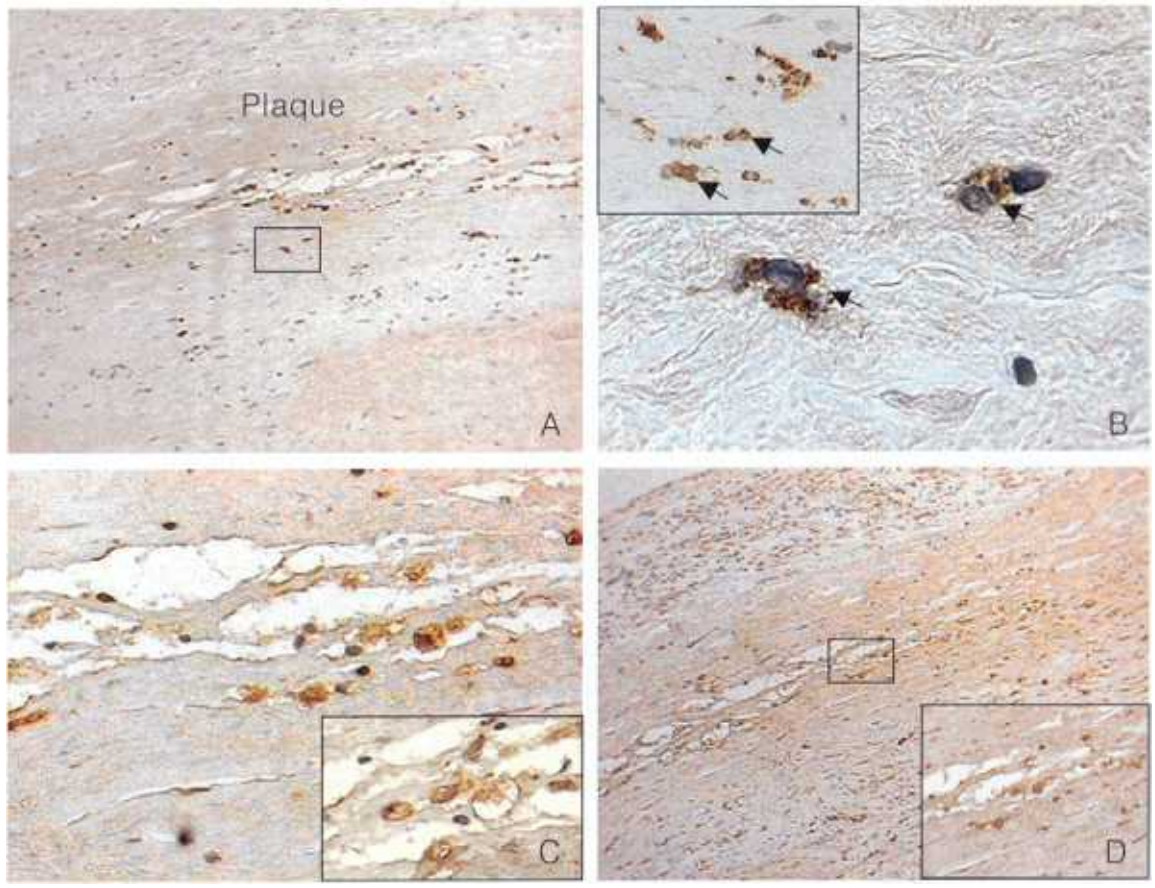


*Fig. 1. Intracellular C. pneumoniae are distributed in the base of atherosclerotic plaque (panel A, ×100) and the immunoreactivity to C. pneumoniae is primarily located in the macrophages/ mononuclear cells (panel B, ×1000). The small box in panel B indicates the macrophage-rich region sampled from panel A and put into a high power view to define colocalization between intracellular C. pneumoniae and tissue macrophages/mononuclear cells (small box in panel B, ×400, immunostaining with HAM56). The arrow in panel B indicates macrophages (HAM56+) that are stained positively to C. pneumoniae. Expression of MMP-9 (panel C, ×400), COX-2 (small box in panel C, ×400), and TIMP-1 (panel D, ×100 and ×400 in the small box) show colocalization of immunoreactivity between C. pneumoniae, COX-2, MMP-9 and its inhibitor (TIMP-1).*

munoreactivity in COX-2, MMP-9 and its inhibitor (TIMP-1) was colocalized to the area stained with *C. pneumoniae* (Fig. 1).

## DISCUSSION

Atherosclerosis and its related diseases, in particular myocardial infarction (MI) and coronary heart disease (CHD), are a major cause of morbidity and mortality worldwide. The differences in the prevalence of conventional cardiovascular risk factors (such as smoking, hypertension, diabetes mellitus, and hypercholesterolemia) do not fully account for the variations in the prevalence or severity of CHD. Consequently, there is intense research interest focused on seeking other atherogenic risk factors.

Current data supports the hypothesis that atherosclerosis is an inflammatory disease rather than a degenerative disease owing to hemodynamic loading,[1,2] and studies examining markers of inflammation (e.g., C-reactive protein) demonstrate a relationship between an increasing inflammation and the risk of a vascular event. It is also recognized that 'plaque activity' and the function of the cellular components can be a more important determinant of the clinical manifestations of atherosclerosis than the degree of stenosis of the arterial lesions. Although many factors

may initiate atherogenesis, the process ultimately involves an inflammatory state in which macrophages and T lymphocytes play a major role.[3-6]

There is a growing amount of evidence to support an association between infection and atherosclerosis, with the first suggestion of a link being made by Osler early in this century.[14] Since Saikku et al first reported that chronic *C. pneumoniae* infection is as a risk factor for coronary heart disease,[12] a number of seroepidemiologic studies have shown a positive relationship between *C. pneumoniae* infection and atherosclerosis.[8-15] The results of our seroepidemiologic study also demonstrated a positive association between *C. pneumoniae* infection and CHD, particularly in the subgroups that had little conventional coronary risk factors, so we suggest that *C. pneumoniae* may play a pathogenetic role in atherosclerosis in low risk groups. Although the microimmunofluorecent (MIF) method is usually used in serologic studies, ELISA is simple and can perform tests for many cases simultaneously. Compared with MIF, the sensitivity and specificity of the ELISA for IgG were 90.4% and 89.9% respectively and 84.6% and 86.7% for IgA, when the MIF titer with more than 1 : 16 was regarded as positive. Therfore, ELISA may be a very useful method for seroepidemiologic study for *C. pneumoniae*.[16] Other limitations of our serologic study were that we did not match in selection of the negative control subjects and did not consider socioeconomic and seasonal factors. To decrease the bias, we subgrouped the subjects by conventional coronary risk factors and analyzed by logistic regression.

In addition to seroepidemiologic studies, a possible etiologic link has been widely investigated and documented through demonstration of an organism in atherosclerotic plaques using various techniques such as immunohistochemistry (IHC), polymerase chain reaction (PCR), electron microscopy and isolation in tissue culture.[17-20] Generally, IHC finds more evidence for *C. pneumoniae* than PCR, although it is not known whether this is due to better sensitivity or worse specificity. These uncertainties make it difficult to estimate the prevalence of *C. pneumoniae* in blood vessels, particularly as specimens positive according to one technique were not necessarily positive by another.[21] In addition, although some seroepidemiologic and histologic data[22,23] has supported an association between *C. pneumoniae* infection and atherosclerosis, the pathogenetic mechanism is not yet clear. Therefore, the aim of our histologic study was not to determine the prevalence of *C. pneumoniae* in atheromatous plaque but to evaluate the possible pathogenetic role of *C. pneumoniae* on atherosclerosis. However, it is difficult to resolve whether it is a primary cause of the disease or whether it is a secondary invader, and if the latter, whether it behaves innocently or aggressively.[24-28]

As mentioned above, vascular inflammation and a chronic degenerative process are prerequisite for atherosclerotic aorto-occlusive and aortic aneurysmal disease. NF-kB, a ubiquitous transcription factor of particular importance in immune and inflammatory responses, increases the expression of the genes for many cytokines, enzymes, and adhesion molecules in chronic inflammatory diseases. This increased expression is reflected by an increased amount of nitric oxide, which has a cytotoxic effect on vascular tissue. Cyclooxygenase-2 (COX-2), another inducible enzyme regulated by NF-kB, is responsible for the increased production of prostagrandins (PG) and thromboxane (TXA) in inflammatory diseases.[29] Two types of COX isoforms have been identified, referred to as COX-1 and COX-2. In contrast of COX-1 which is a constitutively expressed enzyme involved in maintaining low levels of PG, COX-2 is induced in response to cell activators such as growth factors, cytokines, and phorbol esters, suggesting that this enzyme is involved in the generation of PG in inflammation. The induction of COX-2 in monocytes and the resulting production of $PGE_2$ have been shown to be involved in the signal transduction pathway leading to MMPs production in those cells.[30,31] Induced COX-2 expression in monocyte-macrophages and fibroblasts results in increased synthesis of $PGE_2$ and $TXA_2$, which mediate inflammatory change, vasoconstriction, and platelet aggregation. The increased synthesis of COX-2 is considered to play an important role in inflammation and tissue injury. COX-2, which is induced in many cell types in response to cytokines, metabolizes membrane phospholipid arachidonic acid and plays a role in the expression of an inflammatory mediator in heat failure.[31] Gelatinase-B, also known as 92-KD gelatinase or MMP-9, may contribute importantly to the instability of human atherosclerotic plaques. The regulation of transcription of MMP-9 depends in part on a NF-kB elelment in its promoter sequence. This transcription factor is known to be regulated by oxidative stress and may link the accu-

mulation of oxidized lipoproteins in the intima to expression of this particular protease.[32] This study was designed to test the hypothesis that the immunoreactivity for *C. pneumoniae* and inflammatory mediators such as COX-2 and MMP-9 will be colocalized to inflammatory cells in atheromatous plaque if *C. pneumoniae* has some pathogenetic role on atherosclerotic diseases, including aorto-occlusive disease and aortic aneurysmal disease. That is, if the vascular infection of *C. pneumoniae* could induce a chronic inflammatory reaction in host vascular tissue and activate inflammatory cells, some inflammatory mediators such as COX-2 and MMP-9 may exhibit increased expression surrounding macrophages, which have key role in atherosclerosis, infected with *C. pneumoniae.* The results of this study demonstrated that COX-2 and MMP-9 were colocalized to the inflammatory infiltrates of diseased tissues, particularly within *C. pneumoniae*-stained macrophage/monocytes. These results suggest the assertion that *C. pneumoniae* in atheromatous plaque may rather have some pathogenetic role in atherosclerosis than exist as an innocent bystander.

We evaluated the association between *C. pneumoniae* and atherosclerosis indirectly by seroepidemiologic study and suggest here a possible pathogenetic mechanism of *C. pneumoniae* infection on atherosclerosis by histopathologic method. Although it is uncertain whether *C. pneumoniae* is involved in the beginning of atherogenesis or the progression of its disease, this study supported the assertion that *C. pneumoniae* has at least some pathogenetic role in atherosclerosis. The present study may open a promising perspective concerning future therapeutic trials of chronic inflammation related to atherogenesis under pathophysiological conditions.

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