

Endothelial Dysfunction

Association of *Helicobacter pylori* Infection With Systemic Inflammation and Endothelial Dysfunction in Healthy Male Subjects

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OBJECTIVES	The goal of the present study was to determine whether seropositivity to <i>Helicobacter pylori</i> (HP), <i>Chlamydia pneumoniae</i> (CP), and cytomegalovirus (CMV) is associated with systemic inflammation and endothelial dysfunction in healthy male subjects.
BACKGROUND	Chronic infection with certain bacteria and viruses may play an important role in inflammation as the pathogenesis of atherosclerosis.
METHODS	The serum levels of immunoglobulin G antibodies to HP, CP, CMV, high-sensitivity C-reactive protein, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 were determined in 81 healthy Japanese men (40 ± 10 years of age). High-frequency ultrasonographic imaging of the brachial artery was used to study endothelium-dependent (flow-mediated vasodilation) and endothelium-independent (nitroglycerin-induced) vasodilation.
RESULTS	Prevalences of seropositive antibodies to HP, CP, and CMV were 67.9%, 61.7%, and 56.8%, respectively. Infection with HP, CP, or CMV had no relationship with age, blood pressure, or level of serum glucose, lipid, or soluble vascular cell adhesion molecule-1. The levels of C-reactive protein and soluble intercellular adhesion molecule-1 were significantly higher, and flow-mediated vasodilation was significantly lower in subjects with seropositive antibodies to HP than in subjects with seronegative antibodies to HP. Endothelium-independent vasodilation was similar in both groups.
CONCLUSIONS	Chronic infection with HP may be involved in the development of the atherosclerosis via endothelial dysfunction and systemic and vascular inflammation. (J Am Coll Cardiol 2005; 45:1219–22) © 2005 by the American College of Cardiology Foundation

Many observational studies have indicated that an elevated level of C-reactive protein (CRP), a sensitive marker of systemic inflammation, is independently associated with future risk of coronary heart diseases and stroke (1), supporting the hypothesis that inflammation plays an important role in the pathogenesis of atherosclerosis (2). Leukocyte binding to cellular adhesion molecules on the surface of vascular endothelium in response to many inflammatory cytokines and CRP may be the earliest event in vascular inflammatory process (2). These circulating molecules, such as soluble intercellular adhesion molecule (sICAM)-1 and soluble vascular cell adhesion molecule (sVCAM)-1, may serve as markers of endothelial activation and vascular inflammation (3).

Much attention has been given to the possibility that certain infectious agents predispose a person to inflammation and atherosclerosis (4). Many seroepidemiologic stud-

ies have shown associations between previous infections with *Helicobacter pylori* (HP) (5,6), *Chlamydia pneumoniae* (CP) (7), and cytomegalovirus (CMV) (8) and the presence of atherosclerosis in coronary, carotid, or peripheral vessels.

According to the response-to-injury hypothesis of atherosclerosis, endothelial dysfunction is the first step in atherosclerosis (2). This hypothesis has been supported by many studies, indicating that endothelial dysfunction occurs in subjects with classic risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, and smoking (9). If infectious agents are involved in the pathogenesis of atherosclerosis, a significant association may exist of endothelial dysfunction with markers of infection or inflammation. Therefore, the aim of the present study was to determine whether seropositivity to HP, CP, and CMV is associated with elevated levels of CRP, sICAM-1, and sVCAM-1 and endothelial dysfunction in healthy subjects without established risk factors for atherosclerosis.

METHODS

Subjects. The subjects comprised 81 Japanese men who were recruited from staff of the Hiroshima University Graduate School of Biomedical Sciences and their families,

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Manuscript received August 4, 2004; revised manuscript received December 8, 2004, accepted January 4, 2005.

Abbreviations and Acronyms

CMV	= cytomegalovirus
CP	= <i>Chlamydia pneumoniae</i>
CRP	= C-reactive protein
FMD	= flow-mediated vasodilation
HP	= <i>Helicobacter pylori</i>
IgG	= immunoglobulin G
sICAM	= soluble intercellular adhesion molecule
sVCAM	= soluble vascular cell adhesion molecule

friends, and volunteers. All subjects were asymptomatic, lifelong nonsmokers, had no clinical evidence of cardiovascular disease, other serious diseases, hypertension, diabetes mellitus, or hyperlipidemia. This study was approved by the Ethics Committee of the Hiroshima University Faculty of Medicine. Informed consent for participation in the study was obtained from all subjects.

Laboratory analyses. Routine chemical methods were used to determine serum concentrations of cholesterol, triglyceride and glucose, and hemoglobin A1c. Serum levels of CRP were measured by a highly sensitive nephelometric assay using a monoclonal antibody to CRP (Dade Behring, Deerfield, Illinois) with the lowest detection point of 0.2 mg/l. Serum levels of sICAM-1 and sVCAM-1 were determined by commercially available enzyme-linked immunosorbent assays (R & D Systems, Minneapolis, Minnesota).

Commercially available kits were used to determine serum immunoglobulin G (IgG) antibodies to infectious agents. The HP-specific IgG was measured by an enzyme-linked immunosorbent assay kit (Enteric Products Inc., Westbury, New York). Enzyme-linked immunosorbent assay kits were used to determine serum IgG antibodies to CMV (Denka Seiken Co., Ltd., Tokyo, Japan). Serum IgG antibody against CP were determined by an enzyme-linked immunosorbent assay using chlamydial outer membrane complex as an antigen (Hitachi Chemical Co., Ltd, Tokyo, Japan). Seropositivity was decided according to the recommendation of the manufacturers.

Ultrasound studies of the brachial artery. High-resolution ultrasonography (Philips, SONOS 550, Amsterdam, the Netherlands) and a broad-band (8- to 15-MHz) linear array transducer were used to measure changes in arterial diameter in response to increased flow (flow-mediated vasodilation [FMD]) and to sublingual nitroglycerin spray (400 μ g). The brachial artery 2 to 10 cm above the antecubital fossa was scanned in longitudinal section. Increased flow was taken by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg followed by deflation after 5 min. Flow was calculated from Doppler flow velocity and vessel diameter. Reactive hyperemia was calculated as the maximum flow after cuff deflation. The intraobserver coefficient of variation was 3.0% for FMD and 3.5% for nitroglycerin response.

Statistical analysis. All data are expressed as means \pm SD. Differences between means were compared by the Mann-Whitney *U* test. Categorical data were analyzed by the chi-square test. A *p* value <0.05 was considered statistically significant. Univariate correlations were performed using Pearson's correlation coefficient.

RESULTS

Characteristics of subjects. Eighty-one men were enrolled in this study. The average age was 40 ± 10 years. Mean body mass index was 21.8 ± 1.2 kg/m². Mean systolic and diastolic blood pressure was $118 \pm 10/72 \pm 8$ mm Hg. Mean lipid values were as follows: total cholesterol, 4.7 ± 0.6 mmol/l; low-density lipoprotein, 2.5 ± 0.4 mmol/l; high-density lipoprotein, 1.6 ± 0.3 mmol/l; and triglycerides, 1.3 ± 0.3 mmol/l. Fasting glucose was 5.6 ± 0.4 mmol/l, and hemoglobin A1c was $5.2 \pm 0.3\%$. Mean CRP was 1.92 ± 0.7 mg/l, sICAM-1 was 322 ± 76 ng/ml, and sVCAM-1 was 417 ± 99 ng/ml. The prevalences of seropositivity to HP, CP, and CMV were 67.9%, 61.7%, and 56.8%, respectively. Five subjects were negative for all three pathogens. Twenty-one subjects had previous exposure to one pathogen, 34 to two pathogens, and 21 to three pathogens.

The basal diameter of the brachial artery was 4.1 ± 0.4 mm, FMD was $9.6 \pm 2.0\%$, and the response to nitroglycerin was $12.6 \pm 1.8\%$. Basal blood flow was 43 ± 16 ml/min, and hyperemic blood flow was 260 ± 41 ml/min.

Infection, inflammation, and laboratory variables. Baseline characteristics stratified by seropositivity status to HP are summarized in Table 1. No significant difference was found in age, blood pressure, serum lipid, glucose metabolisms, or sVCAM-1 between HP-seropositive and -seronegative groups. The levels of CRP and sICAM-1 were significantly higher in subjects seropositive to HP than in subjects seronegative to HP. Prevalences of seropositivity to CMV and CP were similar in the HP-seropositive and -seronegative groups. No difference was found in each parameter between subjects seropositive and seronegative to CMV or CP. Age was not correlated with the infectious burden.

Normal reference laboratory values of CRP in Hiroshima University Hospital are <1.3 mg/l. The frequency of positive antibodies to HP was significantly greater in 30 subjects with high CRP (≥ 1.3 mg/l) than in 51 subjects with normal CRP (<1.3 mg/l; 90% vs. 55%, *p* < 0.003). Prevalences of seropositivity to CMV (60% vs. 55%) and CP (66% vs. 59%) were similar in the groups with high CRP and normal CRP. Serum level of high-sensitivity CRP was positively and significantly correlated with the titer of antibodies to HP (*r* = 0.34, *p* < 0.01) but not to that to CMV or CP, or the total infectious burden. Serum CRP was significantly correlated with serum sICAM-1 (*r* = 0.37, *p* < 0.01) but not with sVCAM-1.

Infection and vascular function. The FMD vasodilation was significantly lower in subjects seropositive to HP than in

Table 1. Distribution of Variables According to Seropositivity to *Helicobacter pylori*

	HP	HP
	Seronegative	Seropositive
Number	26	55
Age (yrs)	40 ± 12	41 ± 15
Systolic blood pressure (mm Hg)	117 ± 9	119 ± 11
Diastolic blood pressure (mm Hg)	73 ± 7	71 ± 8
Total cholesterol (mmol/l)	4.6 ± 0.5	4.7 ± 0.7
LDL (mmol/l)	2.4 ± 0.4	2.5 ± 0.5
HDL (mmol/l)	1.5 ± 0.3	1.6 ± 0.4
Triglycerides (mmol/l)	1.3 ± 0.3	1.3 ± 0.4
Fasting glucose (mmol/l)	5.7 ± 0.4	5.5 ± 0.5
Hemoglobin A1c (%)	5.2 ± 0.3	5.2 ± 0.3
CRP (mg/l)	1.1 ± 1.1	2.3 ± 2.3*
sICAM-1 (ng/ml)	220 ± 62	370 ± 89*
sVCAM-1 (ng/ml)	412 ± 102	420 ± 92
Seropositivity to CP (%)	64.3	61.5
Seropositivity to CMV (%)	58.9	61.5

*p < 0.05 vs. seronegative.
 CMV = cytomegalovirus; CP = *Chlamydia pneumoniae*; CRP = C-reactive protein; HDL = high-density lipoprotein; HP = *Helicobacter pylori*; LDL = low-density lipoprotein; sICAM = soluble intracellular adhesion molecule-1; sVCAM = soluble intracellular adhesion molecule-1.

seronegative subjects (Table 2). No difference in FMD was found between subjects seropositive and seronegative to CMV or CP. No significant difference in basal or peak hyperemic blood flow was found between subjects divided by seropositivity status to each pathogen. Basal diameter of the brachial artery and its response to nitroglycerin were similar in subjects with and without infection with each pathogen. The FMD or the response to nitroglycerin was not correlated with age, body mass index, blood pressure, serum lipids, serum glucose, hemoglobin A1c, serum CRP, sICAM-1, sVCAM-1, or the total infectious burden.

DISCUSSION

Seroepidemiologic studies have shown that atherosclerosis is associated with several infectious pathogens, including HP (5,6), CP (7), and CMV (8). In addition, CRP, a sensitive marker of systemic inflammation, is well recognized as an independent predictor of coronary heart diseases (1). In the present study, we found that only seropositivity to HP of the

three infections markers tested was associated with elevated CRP. C-reactive protein was elevated in subjects seropositive to HP. Prevalence of seropositivity to HP was significantly increased in subjects with high CRP. Furthermore, serum CRP level was positively correlated with anti-HP antibody titer level. Therefore, chronic infection with HP may account for, at least in part, the elevated serum CRP level in Japanese subjects. Circulating soluble form of ICAM-1, which is expressed on the activated endothelium in response to inflammatory cytokines, also was elevated in subjects seropositive to HP. Chronic infection with HP may be associated with systemic and vascular inflammation.

The second finding in the present study was an association of HP infection with endothelial dysfunction. FMD was attenuated in subjects seropositive to HP. This association remained significant after adjusting for age, body mass index, blood pressure, serum lipids, glucose, and CRP. The basal diameter and reactive hyperemia in the brachial artery were similar in both groups, indicating no difference in the flow stimulus. In subjects with seropositivity to HP, endothelium-independent vasodilation was preserved. This finding indicates that infection with HP may have no effect on the vascular smooth muscle function.

Only a few clinical studies have been published on the association between endothelial function and infection with HP. Prasad et al. (10) reported that attenuation of the coronary vasodilator response to acetylcholine, an index of endothelial function, was observed in subjects seropositive to HP but not in subjects seropositive to CMV or CP. This finding is similar to our finding. However, Khairy et al. (11) reported that no association was found between chronic infection with CP, CMV, or HP and FMD in healthy young men. Because their population was much younger (29.3 ± 5.5 years), the low seroprevalence value for HP (14.3%) may fail to detect a true effect on endothelial function. Grahame-Clarke et al. (12) reported that the response of forearm blood flow assessed by venous occlusion plethysmography to bradykinin and glyceryl trinitrate were attenuated in young diabetic patients with CMV seropositivity but was not affected by positive serology for HP or

Table 2. Vascular Function by Infectious Agent

Infectious Agent	Basal Diameter (mm)		FMD (%)		NTG (%)	
	(-)	(+)	(-)	(+)	(-)	(+)
	HP	4.12 ± 0.44	4.08 ± 0.40	11.8 ± 2.6	8.9 ± 2.0*	12.6 ± 1.8
CP	4.10 ± 0.50	4.10 ± 0.44	9.6 ± 1.9	9.7 ± 1.2	12.2 ± 1.8	13.0 ± 2.6
CMV	4.07 ± 0.40	4.13 ± 0.49	9.5 ± 2.1	9.9 ± 2.0	12.4 ± 1.6	12.8 ± 1.8

	Basal Blood Flow (ml/min)		Hyperemic Blood Flow (ml/min)	
	(-)	(+)	(-)	(+)
	HP	42 ± 15	44 ± 16	252 ± 41
CP	43 ± 20	43 ± 18	257 ± 48	262 ± 40
CMV	42 ± 14	45 ± 22	256 ± 33	266 ± 42

*p < 0.05 vs. (-).
 CMV = cytomegalovirus; CP = *Chlamydia pneumoniae*; FMD = flow-mediated vasodilation; HP = *Helicobacter pylori*; NTG = nitroglycerin; (+) = seropositive; (-) = seronegative.

CP. Their findings may be limited to patients with diabetes.

There are several possibilities for the mechanism underlying a causal role of HP infection in endothelial dysfunction. First, HP may have the direct effect on the structure and function of vascular endothelial cells. Extract of HP has been reported to induce a disturbance of proliferation and apoptosis and to decrease viability of cultured vascular endothelial cells (13). The second possibility is the nutritional effect of HP (14). An infection from HP may cause malabsorption of folate, vitamin B₆, and vitamin B₁₂. This nutritional defect could lead to failure of methylation by 5-methyl-tetrahydrofolic acid and, thus, to hyperhomocysteinemia, which is toxic to endothelial cells.

Because we could not find any relation of previous infection with CMV or CP to CRP, sICAM-1, FMD or vascular dilatatory function, these infectious agents may not play an important role in chronic inflammation or dysfunction of the vascular endothelium or smooth muscle. However, many studies have shown significant association of CMV and CP infections with increased risk of myocardial infarction, coronary death, and stroke (5,8,9). One of the possible reasons for this discrepancy is the difference in studied subjects or race. Because our studied subjects were healthy, these pathogens may interact with classical risk factors rather than having independent effects or might play a role in the later stages of atherosclerosis.

Study limitations. Several other infectious agents are associated with endothelial dysfunction, inflammation, and atherosclerosis. These include Epstein-Barr virus, herpes simplex virus, and hepatitis A virus, among others (15). Because these microorganisms were not included in the present study, there is a possibility that we could not find the impact of total pathogen burden.

Conclusions. We demonstrated high levels of serum CRP and sICAM-1 and attenuated FMD in healthy Japanese men who were seropositive to HP in comparison with those in seronegative subjects. Seropositivity to CMV or CP was not related to these parameters. We hypothesize that chronic infection with HP directly or indirectly induces a persisting systemic and vascular inflammation and endothelial dysfunction.

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