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Infections, Inflammation, and the Risk of Coronary Heart Disease

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Background—The role of infections and inflammation in the pathophysiology of coronary heart disease is emerging. We studied the independent and joint effects of these 2 components on coronary risk.

Methods and Results—We measured baseline levels of C-reactive protein (CRP) and antibodies to adenovirus, enterovirus, cytomegalovirus, and herpes simplex virus as well as to *Chlamydia pneumoniae* (*Cpn*) and *Helicobacter pylori* in 241 subjects who suffered either myocardial infarction or coronary death during the 8.5-year trial in the Helsinki Heart Study, a coronary primary prevention trial. The 241 controls in this nested case-control study were subjects who completed the study without coronary events. Antibody levels to herpes simplex type I (HSV-1) and to *Cpn* were higher in cases than in controls, whereas the distributions of antibodies to other infectious agents were similar. Mean CRP was higher in cases (4.4 versus 2.0 mg/L; $P < 0.001$), and high CRP increased the risks associated with smoking and with high antimicrobial antibody levels. The odds ratios in subjects with high antibody and high CRP levels were 25.4 (95% CI 2.9–220.3) for HSV-1 and 5.4 (95% CI 2.4–12.4) for *Cpn* compared with subjects with low antibody levels and low CRP. High antibody levels to either HSV-1 or to *Cpn* increased the risk independently of the other, and their joint effect was close to additive.

Conclusions—Two chronic infections, HSV-1 and *Cpn*, increase the risk of coronary heart disease. The effect is emphasized in subjects with ongoing inflammation, denoted by increased CRP levels. (*Circulation*. 2000;101:252-257.)

Key Words: infection ■ inflammation ■ proteins ■ coronary disease

Inflammation¹ and infections are involved in the pathogenesis of atherosclerosis and myocardial infarction (MI). Macrophages and T lymphocytes accumulate in atherosclerotic lesions,² and elevated levels of C-reactive protein (CRP), a systemic marker for inflammation, have been found to predict coronary events in population studies and in individuals with angina.^{3–7}

Previous studies have addressed the possible role of infectious agents in the pathogenesis of atherosclerosis and coronary heart disease (CHD). *Chlamydia pneumoniae* (*Cpn*), a human respiratory pathogen, is the bacterium most often found to be associated with CHD, whereas the role of *Helicobacter pylori* (*Hp*), the causative agent of peptic ulcer, is more contradictory.^{8,9} In addition, dental infections have been connected to CHD.¹⁰ Viral agents involved include adenoviruses, coxsackieviruses, and representatives of the Herpesviridae.^{11,12} Adenoviruses cause myocarditis, but no data relate these agents to coronary artery disease. Coxsackie B viruses have been shown to

cause coronary arteritis in experimental animals,¹³ but the association with human CHD has not been confirmed.^{14–19} Our recent study,²⁰ however, discloses an association between high levels of antibodies to enteroviruses, measured by use of a group-specific antigen, and the risk of MI. Cytomegalovirus (CMV) is the herpesvirus most strongly associated with CHD and atherosclerosis.^{9,11,21–23} Herpes simplex 1 (HSV-1) and HSV-2 viruses are found in atherosclerotic lesions,^{24,25} and these agents have also been found in tandem with CMV in these lesions in young trauma victims.²⁶

In this article, we report the association between serological evidence of infections and the risk of MI or coronary death using a nested case-control design among dyslipidemic middle-aged men participating the Helsinki Heart Study,²⁷ a coronary primary prevention trial. The major emphasis was laid on the study of interactions between various infectious agents and chronic inflammation, as indicated by elevated CRP levels.

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TABLE 1. Baseline Characteristics of the Study Population

	Cases (n=241)		Controls (n=241)		P
	Mean	SD	Mean	SD	
Age, y	48.5	4.6	47.2	4.9	<0.01
Systolic blood pressure, mm Hg	148	16	141	16	<0.001
Cholesterol, mmol/L	7.68	0.93	7.47	0.80	<0.01
HDL cholesterol, mmol/L	1.15	0.25	1.26	0.29	<0.001
Triglycerides, mmol/L	2.42	2.18	2.02	1.40	<0.02
Smokers, n (%)	140 (58)		87 (36)		<0.01

Methods

Study Population

The design, methodology, and results of the Helsinki Heart Study have been described previously.^{27,28} Briefly, this was a randomized, placebo-controlled, coronary primary prevention trial with gemfibrozil in dyslipidemic (non-HDL cholesterol >5.2 mmol/L) middle-aged men. Subjects with a history or ECG evidence of CHD or any other major illness were excluded from the study. During the 8.5-year follow-up, 241 out of 4081 study participants suffered either MI or coronary death. These are the CHD cases of the present nested case-control study; the controls were selected from participants who completed the follow-up without CHD. The controls were matched for treatment group (gemfibrozil/placebo) and place of residence. The matching for geography was considered essential because of regional exposure of some infectious agents studied.

Baseline serum samples stored at -20°C were used in the assessment of CHD risk. Baseline sera were available for the analysis in 239 case-control pairs. The samples were analyzed blinded, and the case and the control were always analyzed in the same assay set.

Laboratory Methods

The matched serum pairs were tested for viral IgG antibodies at a dilution of 1:1000 on microtiter plates, each of which also contained positive and negative controls. The results obtained with test sera were expressed as relative units in relation to the standard positive sample.

Enterovirus-group-specific antibodies were measured by a recently developed enzyme immunoassay (EIA) as previously described.²⁰ This assay is based on a synthetic peptide derived from an immunodominant region of the capsid protein VP1, known to be a common antigenic determinant for several different enteroviruses.²⁹ This peptide (KEVPALTA VETGATC with single-letter codes) has been used successfully as a group antigen in serological diagnosis of acute enterovirus infections.^{30,31}

Adenovirus-specific antibodies were measured by EIA with purified hexon of adenovirus type 5 as a group-specific antigen.²⁰

Antibodies to HSV-1 and CMV were measured with commercially available assay kits (Labsystems, catalog No. 6110400 for HSV-1 and 6103201 for CMV). The principles of both assays are based on an indirect solid-phase EIA with horseradish peroxidase or alkaline phosphatase as conjugate enzymes.

Cpn-specific IgA serum antibodies and specific immune complex-bound IgG antibodies were measured by the microimmunofluorescence method with *Cpn* Kajaani 6 strain as antigen, as described in detail earlier.^{32,33} The diagnostic criteria for *Cpn* infection consisted of either IgA antibody titer >40 or immunocomplex-bound antibody titer >2, representing approximately the highest quartile and the median, respectively.

Hp-specific serum IgG antibodies were determined by EIA (Py-loriset, Orion Diagnostic) according to the manufacturer's instructions.

Serum samples were analyzed in duplicate for CRP levels with a sandwich enzyme immunoassay (UC CRP ELISA, Eucardio Labo-

ratory). The limit of detection in this assay is 0.35 mg/L. The reported intra-assay and interassay coefficients of variation for a serum with high CRP level were 3.9% and 8.5% and for a serum with low CRP level, 9.2% and 10.8%, respectively. It is expected that 95% of normal sera have CRP <2 mg/L. CRP levels >3.8 mg/L, corresponding to the highest quartile of distribution, were considered "high."

Statistical Analyses

The differences between cases and controls in continuous risk factors were tested with an unpaired *t* test. A logarithmic transformation was used for variables with skewed distributions (ie, triglycerides). The Mann-Whitney test was applied in class variables. The 241 CHD cases and 241 controls were matched for treatment group (gemfibrozil/placebo) and geographical area of residence. The ORs and 95% CIs for the main effects were obtained by fitting conditional logistic regression models and are presented comparing the third and fourth quartiles to the lowest half of distribution. This approach was selected because of skewed distributions and because the risks associated with all antimicrobial antibodies were almost identical in the lowest quartiles of distribution. An essential part of the modeling for the joint effects was the construction of sets of binary indicator variables for the combinations of interest. In these analyses, the upper quartiles were used as cutpoints in dichotomization of the risk factors (high versus low level). All statistical analyses were carried out with the SAS program.

Results

The distribution of the classic CHD risk factors at study baseline in this case-control population is given in Table 1.

Study baseline levels of antibodies to HSV-1 (IgG) and *Cpn* (IgA) and immunocomplex-bound antibodies (IC) were higher in the CHD cases than in the controls, whereas the distributions of the antibody levels to adenoviruses, enteroviruses, and *Hp* were similar in both groups (Table 2). The most striking difference was found in CRP levels (4.44 versus 2.01 mg/L; $P<0.001$), with 37% of the cases having CRP in the highest quartile of distribution (>3.8 mg/L), compared with 13% in the controls.

High levels of antibodies against HSV-1 and *Cpn* increased the risk significantly (Table 3). When controlled for age and smoking, the risks associated with either high *Cpn* IgA or IC alone did not reach statistical significance. The increment with high CRP level was >4-fold (OR 4.59), and there seemed to be a clear dose-response effect ($P<0.01$ for trend). With the lowest CRP quartile used as reference, the ORs were 0.98 (95% CI 0.55–1.75), 1.57 (0.87–2.82), and 3.66 (1.97–6.81) in the second, third, and highest quartiles when adjusted for age and smoking. Further adjustments for blood pressure and HDL cholesterol had only minimal impact. The associ-

TABLE 2. Distributions of CHD Cases and Controls by Antibody Titers and CRP Level at Study Baseline

Quartile Distribution	Cases		Controls	
	n	%	n	%
Adenovirus (IgG)				
1–2	116	50	108	46
3	60	26	65	28
4	58	25	61	26
Enterovirus (IgG)				
1–2	124	52	114	48
3	55	23	59	25
4	59	25	65	27
CMV (IgG)				
1–2	117	50	118	50
3	56	24	62	26
4	63	27	56	24
HSV (IgG)				
1–2	107	46	126	54
3	58	25	60	26
4	69	29	48	21
Cpn				
IgA				
1–2	120	52	134	58
3	50	22	58	25
4	60	26	38	17
IC				
1–2	115	51	144	63
3	49	22	31	14
4	63	28	52	23
Hp (IgG)				
1–2	117	51	111	49
3	51	22	59	26
4	60	26	58	25
CRP				
1–2	79	37	135	63
3	57	27	53	25
4	79	37	27	13

IgA denotes immunoglobulin class, and IC, immunocomplexes.

ations of high levels of HSV-1 antibodies and high CRP with CHD remained significant after adjustment for age and smoking.

The relative risks, adjusted for age, of high levels of antibodies to HSV-1 and *Cpn* in nonsmokers (Figure) were 2.05 (95% CI 1.15–3.67; 73 cases, 72 controls) and 1.44 (0.82–2.33; 49 cases, 40 controls), whereas smoking increased the risks to 3.74 (1.58–8.86; 47 cases, 14 controls) and 4.88 (2.42–9.81; 20 cases, 8 controls), respectively. When high HSV-1 antibody levels and smoking were considered, their joint effect seemed to be close to additive, whereas the risk associated with high *Cpn* antibody levels was mainly confined to smokers. High CRP level in nonsmokers increased the CHD risk significantly, OR 2.32 (95%

TABLE 3. Relative Risks of Coronary Events by Baseline Levels of Viral and Bacterial Antibodies and CRP

	No Adjustments		Adjusted for Age and Smoking	
	OR	95% CI	OR	95% CI
HSV				
1–2 quartile	1		1	
3	1.13	0.74–1.72	1.12	0.71–1.78
4	1.85	1.13–3.04	2.07	1.20–3.56
Chlamydia pneumoniae				
IgA				
1–2 quartile	1		1	
3	0.92	0.59–1.45	0.82	0.51–1.34
4	1.74	1.07–2.83	1.35	0.79–2.33
IC				
1–2 quartile	1		1	
3	1.96	1.19–3.24	1.88	1.10–3.21
4	1.55	0.99–2.42	1.36	0.84–2.22
CRP				
1–2 quartile	1		1	
3	2.17	1.27–3.68	1.70	0.96–3.02
4	4.59	2.68–7.86	3.56	1.93–6.57

IgA and IgG denote immunoglobulin classes, and IC, immunocomplexes.

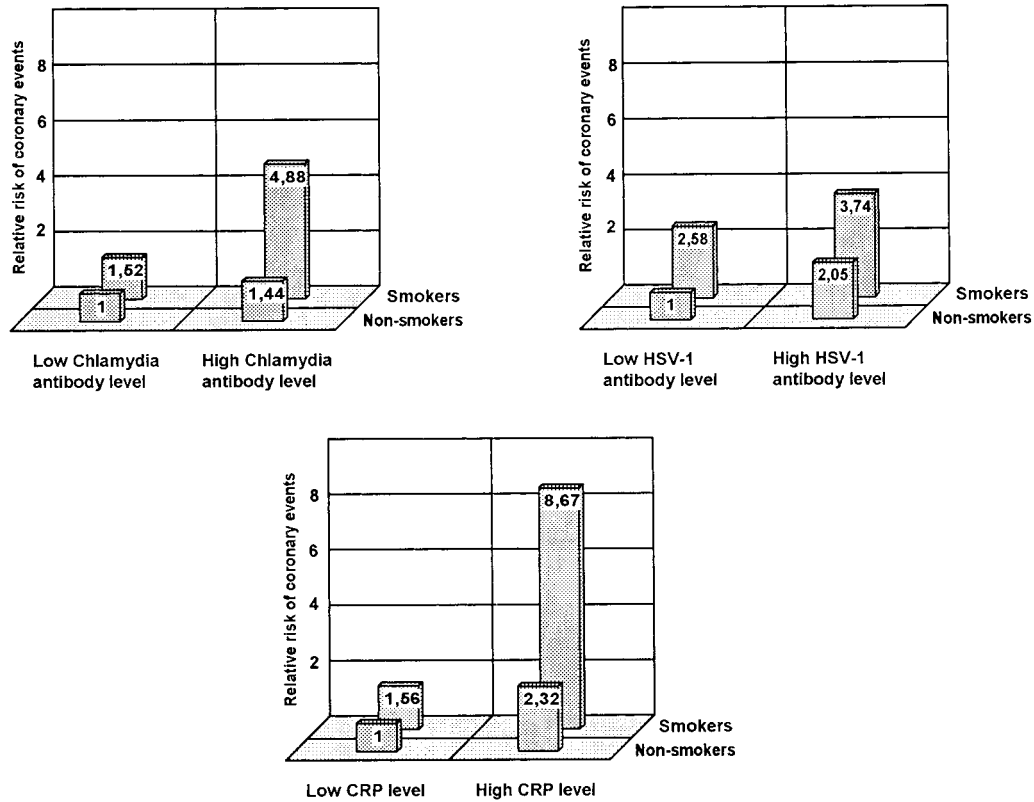
CI 1.27–4.24; 40 cases, 21 controls), whereas the joint effect of high CRP and smoking was associated with a 9-fold increase in risk, OR 8.67 (3.52–21.4; 39 cases, 6 controls) compared with nonsmokers with low CRP (102 cases, 155 controls).

Simultaneous occurrence of high CRP and high HSV-1 antibody levels as well as high CRP and high *Cpn* antibody levels increased CHD risk substantially (Table 4). The simultaneous presence of high HSV-1 and high *Cpn* antibody levels increased the risk compared with the presence of either of these alone. When subjects with low levels of both antibodies were used as reference, ORs were 1.97 in subjects with high HSV-1 only, 1.74 in subjects with high *Cpn* only, and 4.10 in subject with high levels of both antibodies.

Discussion

Our study demonstrated that high levels of antibodies and/or circulating immune complexes against HSV-1 and *Cpn* were risk factors for future coronary events in a prospective cohort of middle-aged dyslipidemic men. No associations were found, however, between CHD and high levels of antibodies against *Hp*, adenovirus, cytomegalovirus, or enterovirus. The risks associated with HSV-1 and *Cpn* were strongly modified by CRP, a serological marker of chronic inflammation, and by smoking.

In this study, we considered high IgG antibody levels as markers of previous infections. All microbes studied are common, however, and in addition to acute infections, most of them also cause chronic and/or latent infections. It may well be, as suggested by the data of Pesonen,³⁴ that a process that eventually leads to CHD is initiated in early life after infections acquired in childhood. However, assessment of the



Joint effects of antimicrobial antibodies, smoking, and CRP on the risk of coronary events.

chronicity of an infection is a complicated issue, and it is not clear whether increased levels of IgG antibodies reflect the duration of the infection, reactivation of a latent infection, reinfection, or some unknown immunological features of the host. There is evidence, however, for the association of increased HSV antibody levels and a history of frequent herpes recurrences.³⁵ With regard to *Cpn* antibodies, conversely, the persistent presence of elevated IgA titers and specific immune complexes has been shown to reflect chronic *Cpn* infection.³⁶

Our results are in agreement with previous data demonstrating an association between *Cpn* and CHD.^{8,9} It should be noted that our previous report of this relation describes a subgroup of 103 cardiac events from a total of 241 reported in this paper.³⁷ The seroepidemiological data relating *Cpn* and CHD are consistent in the majority of the studies, and the presence of the agent has been demonstrated in atherosclerotic lesions.³⁷⁻³⁹ The association between *Hp* and CHD is more controversial, and when it is adjusted for other risk factors, the contribution is minimal.^{9,40} It may be that there are certain virulent strains that are more aggressive in this respect.⁴¹

Viral agents previously implicated in the pathogenesis of CHD include coxsackieviruses and representatives of the Herpesviridae.^{9,11} The problems in the studies of enteroviruses have been the large number of serotypes, the serotype-specific antibody assays, and the epidemic nature of enteroviral disease. Our present findings of no association between high enterovirus antibody levels and CHD are in contrast to the recent data derived from another Finnish cohort.²⁰

HSV-1 and HSV-2 have been found in human atherosclerotic lesions^{24,25} and CMV in restenotic lesions after angioplasty.^{22,23} CMV and HSV have both been found in tandem in early atherosclerotic lesions of young trauma victims.²⁶ Previous seroepidemiological evidence both supports and is contradictory to the concept that CMV or HSV-1 is involved in the pathogenesis of atherosclerosis.⁴²⁻⁴⁴ We found no association between CMV and CHD, but antibody level in the highest quartile of distribution to HSV was a risk factor for future coronary events.

Our finding that a high serum level of CRP, an acute-phase protein used as a marker for inflammation, increases the risk for cardiac events is in accord with previous findings.³⁻⁷ The CRP level in our study cohort was related to the number of cigarettes smoked (data not presented), in agreement with previous studies.^{45,46} The detected interaction on CHD risk between high CRP and smoking might well be one of the pathways between smoking and CHD. Conversely, the finding is in contrast with the data from the Physicians' Health Study.⁴⁷ An interaction in our study cohort was found between high *Cpn* antibody level and smoking, with the risk almost totally confined to smokers, whereas the joint effect of smoking and high HSV antibody level on CHD risk indicated more of an additive effect.

The study of the joint effects between high microbial antibody levels and high CRP disclosed differences between the 2 agents. High HSV level increased the risk even in subjects with low CRP, but to increase the risk, high *Cpn* antibody levels required the presence of high CRP. The independent contribution of both high HSV and high *Cpn*

TABLE 4. Interactions of Microbial Antibodies and CRP on the Risk of Coronary Events

	No. Cases/Controls	No Adjustments		Adjusted for Age and Smoking	
		OR	95% CI	OR	95% CI
HSV-1					
Low CRP—Low HSV-1	84/140	1		1	
Low CRP—High HSV-1	47/42	2.21	1.24–3.94	2.36	1.28–4.36
High CRP—Low HSV-1	62/25	3.50	2.05–5.98	3.10	1.68–5.71
High CRP—High HSV-1	16/2	24.93	3.17–196.0	25.44	2.94–220.3
<i>Cpn</i>					
Low CRP—Low <i>Cpn</i>	70/110	1		1	
Low CRP—High <i>Cpn</i>	59/68	1.29	0.81–2.07	1.22	0.74–2.01
High CRP—Low <i>Cpn</i>	25/15	1.99	1.01–3.93	1.67	0.81–3.42
High CRP—High <i>Cpn</i>	48/9	6.74	3.09–14.72	5.40	2.35–12.43
Any other microbial antibody (MICA)*					
Low CRP—Low MICA	7/6	1		1	
Low CRP—High MICA	18/22	0.60	0.16–2.31	0.55	0.12–2.58
High CRP—Low MICA	4/2	1.64	0.27–10.0	1.70	0.23–12.5
High CRP—High MICA	8/7	0.79	0.17–3.60	0.75	0.13–4.54
HSV-1 and <i>Cpn</i>					
Low HSV-1—Low <i>Cpn</i>	75/113	1		1	
Low HSV-1—High <i>Cpn</i>	80/63	1.98	1.25–3.14	1.74	1.04–2.91
High HSV-1—Low <i>Cpn</i>	27/25	1.79	0.92–3.50	1.97	0.95–4.08
High HSV-1—High <i>Cpn</i>	38/19	3.61	1.78–7.30	4.10	1.88–8.98

High indicates highest tertile, and low, 2 lower tertiles.

*Those with high HSV-1 or *Cpn* excluded.

antibody levels in the joint effect analysis indicates that chronic infection with either of these 2 agents alone increases the risk and that coexistence of the other is close to additive with regard to the CHD risk.

The risk associated with high antibody levels alone were moderate in our study cohort. However, when the CRP level was simultaneously high, the risks were increased substantially. Our results thus support the hypothesis that inflammatory reaction can be one of the major factors in the pathophysiology of atherosclerosis and suggest that at least 2 different infections are capable of triggering this reaction.

Studies like the present one, based on selected populations, obviously have natural shortcomings and restrictions. All participants were dyslipidemic, white, middle-aged men, and the results may not be generalizable to other age groups, to other ethnic populations, to normolipidemics, or to women. Another shortcoming is the post hoc hypothesis, but the results in this kind of study should be considered more as hypothesis-generating. Conversely, the strengths of this study are the homogeneous population and very careful follow-up.

In conclusion, we have shown in this prospective study that high antibody levels to HSV-1 and to *Cpn* as markers of chronic, active, or recurrent infection were associated with an increased risk of CHD, whereas high antibody levels to adenovirus, cytomegalovirus, and enterovirus and to *Hp* were not. The risks associated with high antibody levels were strongly modified by smoking, and the simultaneous occurrence of elevated CRP level substantially increased the CHD

risk. The high antibody levels to HSV-1 or to *Cpn* increased the risk independently of the other, indicating that at least 2 different infections are potential triggers of the inflammatory reaction, one of the key events in atherosclerosis.

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