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Prospective Study of Herpes Simplex Virus, Cytomegalovirus, and the Risk of Future Myocardial Infarction and Stroke

Paul M. Ridker, MD; Charles H. Hennekens, MD; Meir J. Stampfer, MD; Fred Wang, MD

Background—It has been hypothesized that infection with either herpes simplex virus (HSV) or cytomegalovirus (CMV) is associated with atherogenesis. However, prospective data relating evidence of prior exposure to these agents with risks of future myocardial infarction (MI) and stroke are sparse.

Methods and Results—In a prospective, nested case-control study of apparently healthy men, the baseline prevalence of antibodies directed against HSV or CMV was similar among 643 men who subsequently developed a first MI or thromboembolic stroke and among 643 age- and smoking-matched men who remained free of reported vascular disease over a 12-year follow-up period. Specifically, the relative risks for future MI and stroke were 0.94 (95% CI, 0.7 to 1.2) for HSV seropositivity and 0.72 (95% CI, 0.6 to 0.9) for CMV seropositivity, after adjustment for other cardiovascular risk factors. These findings were not materially altered in comparisons of early versus late events or in analyses stratified by smoking status. There was no evidence of association between HSV or CMV antibodies and plasma concentration of C-reactive protein, a marker of inflammation that predicts vascular risk in this cohort.

Conclusions—Among apparently healthy middle-aged men, IgG antibodies directed against HSV or CMV do not appear to be a marker for increased atherothrombotic risk. The observed possible inverse relationship of CMV with MI and stroke was unexpected and may well be due to chance, because the direction of association is not compatible with the a priori hypothesis based on proposed biological mechanisms or previous cross-sectional and retrospective data. (*Circulation*. 1998;98:2796-2799.)

Key Words: infection ■ atherosclerosis ■ viruses ■ myocardial infarction ■ stroke

The hypothesis that infection with herpes simplex virus (HSV) or cytomegalovirus (CMV) may play a role in atherogenesis is supported by several observations.^{1,2} Clinical studies report an increased prevalence of herpetic infection among individuals with accelerated atherosclerosis,³⁻⁵ particularly in the setting of cardiac transplantation.^{6,7} Histopathological studies have reported evidence of HSV and CMV particles within atherosclerotic vessels.^{1,8,9} Furthermore, in avian models, herpes viral infection results in arterial plaques (Marek's disease) similar to human atheroma.¹⁰

Epidemiological data relating herpetic infection with atherosclerotic risk in humans have derived almost exclusively from patients with transplanted hearts,^{6,7} from studies of restenosis after percutaneous coronary atherectomy,^{11,12} or from cross-sectional or retrospective studies in which evidence of exposure to these viruses was ascertained after rather than before the development of

atherothrombosis.¹⁻⁵ By contrast, prospective cohort studies evaluating the prevalence of HSV and CMV antibodies before the onset of myocardial infarction (MI) and stroke are sparse. Thus, whether infection with HSV or CMV has an etiologic role in the development of cardiovascular disease remains uncertain. We therefore tested whether serological responses to these agents were markers of risk for future MI or thromboembolic stroke in a prospective case-control study nested within a large cohort of apparently healthy men who were followed up over a 12-year period.

Methods

We determined the presence or absence of IgG antibodies directed against HSV and CMV among case and control subjects from the Physicians' Health Study (PHS), a randomized trial of aspirin and β -carotene conducted among 22 071 US male physicians with no prior history of MI, stroke, or cancer.¹³ Before randomization, 14 916 participants (68%) provided baseline blood samples, which

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From the Divisions of Preventive Medicine (P.M.R., C.H.H.), Cardiovascular Diseases (P.M.R.), Infectious Diseases (F.W.), and the Channing Laboratory (M.J.S.), Department of Medicine, Brigham and Women's Hospital; the Department of Ambulatory Care and Prevention (C.H.H.), Harvard Medical School; and the Departments of Epidemiology (C.H.H., M.J.S.) and Nutrition (M.J.S.), Harvard School of Public Health, Boston, Mass.

Correspondence to Paul M. Ridker, Brigham and Women's Hospital, 900 Commonwealth Ave E, Boston, MA 02115. E-mail pmridker@bics.bwh.harvard.edu

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TABLE 1. Baseline Clinical Characteristics of Study Subjects

	Cases (n=643)	Controls (n=643)	P
Age, y	58.5	58.2	...
Smoking status, %			
Never	45.0	45.0	
Past	40.7	40.7	...
Current	14.3	14.3	
BMI, kg/m ²	25.5	24.9	0.001
Blood pressure, mm Hg			
Systolic	131.0	126.7	0.001
Diastolic	81.0	79.2	0.001
History of hypertension, %	29.3	15.3	0.001
Total cholesterol, mg/dL	221.3	211.4	0.001
HDL cholesterol, mg/dL	46.1	49.8	0.001
History of hyperlipidemia, %	14.4	9.8	0.02
Diabetes, %	7.5	2.0	0.001
Family history of coronary artery disease, %	15.8	12.2	0.06

were collected in EDTA and stored at -80°C until the time of analysis.

For all cases of MI or thromboembolic stroke reported during the follow-up period, hospital records and autopsy reports were used to confirm each diagnosis according to prespecified criteria as previously described.¹³ Each participant who provided a baseline blood sample for analysis and had a confirmed MI or thromboembolic stroke during follow-up was matched with 1 control participant who also had provided a baseline blood sample but remained free of reported cardiovascular disease at the time the matched case patient reported his event. Control subjects were randomly selected from the pool of PHS participants free of cardiovascular disease who met the matching criteria of age, smoking habit, and length of follow-up. Overall, 643 case-control pairs were included in this analysis.

For each case and control subject, plasma collected and stored at baseline was thawed and assayed by ELISA for the presence or absence of IgG antibodies directed against HSV and CMV (Herpes Group IgG ELISA, Wampole Laboratories, and CMV IgG ELISA, Gull Laboratories). Blood specimens were analyzed in blinded pairs, with the position of the case and control specimens varied at random to reduce the possibility of systematic bias and decrease interassay variability.

We used conditional logistic regression analyses to test any association between the presence of IgG antibodies at baseline and the subsequent development of MI or stroke. All analyses controlled for randomized treatment assignment; adjusted estimates of risk were computed after additional control for baseline differences between case and control subjects. All probability values are 2-tailed, and CIs were computed at the 95% level.

Results

As expected, participants who developed MI or stroke during follow-up (cases) were more likely than controls to have baseline evidence of hypertension, obesity, diabetes, hyperlipidemia, or a family history of premature coronary disease. As a result of the matching, age and smoking status were identical in the 2 groups (Table 1).

Baseline prevalence of HSV seropositivity was virtually identical for case and control subjects (69.0% versus 69.4%), such that the crude relative risk (RR) of any

TABLE 2. Crude and Adjusted RRs of Future Cardiovascular Events According to the Presence of Baseline Antibodies Directed Against HSV and CMV

	Controls (n=643)	All Cases (n=643)	MI Cases (n=372)	Stroke Cases (n=271)
HSV				
% Positive	69.4	69.0	65.6	73.6
RR (crude)	...	0.98	0.92	1.1
95% CI	...	0.8–1.2	0.7–1.2	0.7–1.6
RR (adjusted)	...	0.94	0.88	1.0
95% CI	...	0.7–1.2	0.6–1.2	0.7–1.5
CMV				
% Positive	70.2	63.5	62.1	65.3
RR (crude)	...	0.77	0.72	0.85
95% CI	...	0.6–1.0	0.5–1.0	0.6–1.2
RR (adjusted)	...	0.72	0.74	0.67
95% CI	...	0.6–0.9	0.5–1.0	0.4–1.0

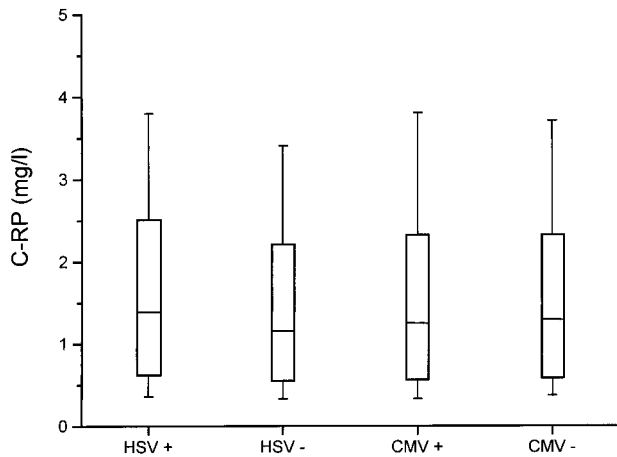
All models matched on age and smoking status and controlled for randomized treatment assignment. Adjusted models further controlled for body mass index (kg/m²), history of hypertension, history of hypercholesterolemia, diabetes, and a family history of premature atherosclerosis. Data are shown for all arterial events (cases) and separately for MI and stroke.

atherothrombotic event associated with prior HSV exposure was 0.98 (95% CI, 0.8 to 1.2) (Table 2, top). No evidence of association between baseline prevalence of HSV seropositivity and risk was found for MI (RR=0.92; 95% CI, 0.7 to 1.2) or for thromboembolic stroke (RR=1.1; 95% CI, 0.7 to 1.6). These risk estimates were not materially changed in multivariate analyses controlling for baseline differences in body mass index, blood pressure, hyperlipidemia, diabetes, or family history of premature atherosclerosis.

Baseline prevalence of CMV seropositivity was also similar between the case and control groups (63.5% versus 70.2%), such that the crude RR for any atherothrombotic event associated with the presence of CMV antibodies was 0.77 (95% CI, 0.6 to 1.0), whereas the RRs for MI and stroke were 0.72 (95% CI, 0.5 to 1.0) and 0.85 (95% CI, 0.6 to 1.2), respectively (Table 2, bottom). After control for other cardiovascular risk factors, the adjusted RR of any atherothrombotic event associated with CMV seropositivity was 0.72 (95% CI, 0.6 to 0.9).

We found no evidence of any positive association for either HSV or CMV and subsequent vascular risk in additional analyses stratified by smoking status, by the presence or absence of other cardiovascular risk factors, or by duration of follow-up (data not shown).

Because elevated baseline levels of C-reactive protein (CRP) have previously been shown to be a marker for future cardiovascular risk in this cohort,^{14–16} we sought to determine whether seropositivity directed against HSV or CMV might be associated with elevated levels of this inflammatory marker. As shown in the Figure, distributions of CRP were similar among those with and without prior exposure to HSV and among those with or without prior exposure to CMV. Furthermore, the distributions of



Distribution of CRP concentration among study participants according to presence or absence of antibodies directed against HSV or CMV.

CRP were similar between those with evidence of prior exposure to both HSV and CMV compared with those with no evidence of exposure to either of these agents ($P=0.2$).

Discussion

In a large cohort of apparently healthy middle-aged American men followed up over a 12-year period, we found no evidence of a positive association between baseline IgG antibodies directed against CMV or HSV and the development of future MI or thromboembolic stroke. Furthermore, we found no evidence of association between CMV or HSV exposure and concentration of CRP.

Although we measured HSV and CMV antibodies on a single plasma sample obtained at study entry, IgG antibodies directed against these agents are stable over long periods of time after exposure, and the presence or absence on a single blood screen is routinely used in clinical testing. In addition, the prevalence of IgG seropositivity among our control group is virtually identical to that reported in several other population-based studies of men of similar age. Furthermore, in pilot data, the presence of IgG antibodies detected in our stored frozen plasma samples was highly correlated with the presence of antibodies detected in fresh plasma samples obtained in a subgroup of study participants. Thus, we believe it unlikely that the blood collection, processing, storage, or assay conditions used in our study could have led to a false-negative result.

The prospective design and long follow-up period, as well as the fact that all study participants derive from a socioeconomically homogeneous study population, all reduce the possibility of bias and confounding in these data. Furthermore, in addition to the matching criteria of age and smoking, we were able to control for a wide variety of other potential confounding factors in our analysis. Thus, in contrast to some prior cross-sectional and retrospective studies evaluating the role of infection in atherosclerosis, the potential for residual confounding in our study is far less.¹⁷ Finally, the large sample size reduces the likelihood that we failed to detect a clinically important positive

association between seropositivity and vascular risk. Specifically, the upper limit of the 95% CI for the adjusted RR for MI or stroke was 1.2 for HSV and 0.9 for CMV. The unexpected observed inverse relationship of CMV seropositivity with MI and stroke may be due to chance and should be viewed in the context that the direction of association is not compatible with either proposed biological mechanisms or previous cross-sectional and retrospective studies.

Despite our null findings, we believe that these data alone cannot exclude a potential role for viral infection in development of CVD. For example, MI and stroke involve not only atherogenesis but also thrombosis, and it is possible that viral particles might be involved in the former process but not the latter. In this regard, it is theoretically possible that herpetic infection might lead to accelerated atherosclerotic progression without necessarily increasing rates of clinical thrombosis; this possibility is consistent with the observation that HSV and CMV particles have been recovered from all segments of the arterial tree, not simply from lesions directly associated with vascular occlusion.^{8,9} Furthermore, experimental data suggest that subacute CMV infection can inhibit the p53 gene product, a process that in turn may lead to cellular proliferation and restenosis^{11,12}; because our study did not address acute infection or evaluate postangioplasty patients, these hypotheses cannot be tested in the present study. Finally, because herpesvirus infections are characterized by latency and reactivation, it has been suggested that CMV and HSV may have roles as chronic inflammatory stimulants contributing to atherogenesis.^{1,2} However, our data show no significant differences in CRP levels between seropositive and seronegative patients who develop atherothrombosis, suggesting that HSV and CMV infection or reactivation is unlikely to play any major role in the inflammatory response and CRP elevations found in this cohort.¹⁴⁻¹⁶

Thus, although the present data indicate that the presence of HSV or CMV antibodies at baseline is not a marker of risk for future MI or stroke, further basic, clinical, and prospective epidemiological data are needed before firm conclusions can be drawn.

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