A Prospective Study of Cytomegalovirus, Herpes Simplex Virus 1, and Coronary Heart Disease

The Atherosclerosis Risk in Communities (ARIC) Study

Paul D. Sorlie, PhD; F. Javier Nieto, MD, PhD; Ervin Adam, MD; Aaron R. Folsom, MD; Eyal Shahar, MD; Mark Massing, MD, MPH

Background: Conflicting evidence exists implicating infectious disease in the pathological processes leading to coronary heart disease (CHD). The objective of this article is to describe the relationship of previous infection with cytomegalovirus (CMV) and herpes simplex virus 1 to incident CHD in a population-based cohort study.

Methods: Using a nested case-cohort design from the Atherosclerosis Risk in Communities Study, antibody levels to CMV and herpes simplex virus 1 were determined in serum samples that had been frozen at the baseline examination in participants free of CHD. Determinations were made in those who developed incident CHD (n=221) during follow-up of up to 5 years from baseline and in a stratified random sample of all participants (n=515).

Results: The population with the highest antibody levels of CMV (approximately the upper 20%) showed an increased relative risk (RR) of CHD of 1.76 (95% confidence interval, 1.00-3.11), adjusting for age, sex, and race. After adjustment for additional covariates of hypertension, diabetes, years of education, cigarette smoking, low-density lipoprotein and high-density lipoprotein cholesterol levels, and fibrinogen level, the RR increased slightly. Based on a priori hypotheses, the RR of CHD at the highest antibody levels in individuals with diabetes was particularly large but with wide confidence intervals (RR, 9.2; 95% confidence interval, 1.8-47.0), and the interaction between high levels of antibody to CMV and diabetes was statistically significant (P=.05). There was no association of CHD with the highest herpes simplex virus 1 antibody levels (adjusted RR, 0.77; 95% confidence interval, 0.36-1.62).

Conclusions: High levels of CMV antibodies are significantly associated with incident CHD. Infection with CMV, particularly in more susceptible disease states such as diabetes, may be an important risk factor for CHD.

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IN A RECENT REVIEW1 of published studies of chronic infection and coronary heart disease (CHD), the results of 18 studies of cytomegalovirus (CMV) and atherosclerosis were presented. Nearly all of these studies used, as their outcome, a measure of arterial wall thickness, stenosis, or restenosis and showed moderate, and not always statistically significant, odds ratios associated with CMV seropositivity of 1.5 to 2.0, although some were higher and some were lower. The biological plausibility of these associations was also reviewed, outlining the potential short-term effects of precipitating a plaque rupture or the long-term effects of initiation and promotion of plaque growth.

Previous studies from the Atherosclerosis Risk in Communities (ARIC) Study have analyzed carotid wall thickness in relation to concurrent measures of CMV and herpes simplex virus (HSV) types 1 and 2,2, with earlier measures of antibody levels, and have investigated the associations of antibody levels with hemostatic factors.3 These studies were included in the review above1 and show moderate associations of CMV antibodies with carotid atherosclerosis. The present analysis uses the ARIC Study to analyze CMV and HSV-1 antibody levels in relation to incident CHD in a population-defined cohort free of CHD. Antibody levels were determined at baseline using frozen serum specimens from a random sample of ARIC participants and in those who developed CHD during follow-up of up to 5 years. Using this nested case-cohort design, the relative risk (RR) of CHD by antibody level was estimated with antibody determinations made in frozen serum specimens obtained up to 5 years before CHD events.
PARTICIPANTS AND METHODS

The ARIC Study is a prospective epidemiological study of CHD and atherosclerosis in sampled populations from 4 US communities (Forsyth County, North Carolina; Jackson, Miss; suburban Minneapolis, Minn; and Washington County, Maryland). Each sample was selected from the entire community aged 45 to 64 years, with the exception of Jackson, which sampled only the African American population in that community. Details of the study design have been published previously.3 Approximately 4000 persons from each community were examined between January 1, 1987, and December 31, 1989; were given another examination every 3 years; and were followed up for morbidity and mortality. The baseline examination consisted of medical history and lifestyle questionnaires; anthropometric measurements; and determination of lipid levels, hemostatic variables, blood chemistries, and hematologic variables. Ultrasound measurement of carotid and popliteal arteries was performed, as were an electrocardiogram and a physical examination. Serum and plasma samples were collected and frozen for future studies. All participants gave informed consent, and the study was approved by local institutional review boards.

Incident CHD cases were determined as occurring during follow-up, between baseline examination and December 31, 1991. Follow-up was a maximum of 5 years. A case was defined as a definite or probable myocardial infarction (including silent myocardial infarctions), a definite CHD death, or a coronary revascularization.4 Participants were contacted annually to identify all hospitalizations and deaths. Vital records and hospital discharge lists were reviewed to detect mortality and morbid events. Out-of-hospital deaths were investigated by interviewing the next of kin and participants’ physicians. Coroner and autopsy reports were used when available. For hospitalized patients, hospital records were abstracted by trained abstractors recording signs, symptoms, cardiac enzyme levels, and related clinical information. Hospital electrocardiograms were coded using the Minnesota Code and were evaluated for waveform evolution using side-by-side comparisons. A classification committee reviewed and adjudicated all potential clinical CHD events using published criteria based on symptoms, electrocardiographic findings, and cardiac enzyme levels for hospitalized cases.6 Coronary revascularization was defined as present when hospital procedure codes included coronary bypass, coronary angioplasty, or coronary atherectomy.

The hypotheses regarding the association of CMV and HSV-1 with CHD were tested using a nested case-cohort study design within the ARIC Study. The case-cohort design and sample selection were chosen early in the ARIC Study so that multiple research projects using stored frozen specimens could be done using a sample size sufficient for most hypotheses. Incident cases of CHD in the ARIC cohort were identified through December 31, 1991 (n=257 before exclusions for missing assays). A sample of the total cohort was also selected (n=356 before exclusions).

Table 1 shows the characteristics of the 221 CHD cases and 505 randomly selected noncases weighted to reflect the entire ARIC Study population. Although not adjusted for confounders, Table 1 shows that CHD cases, compared with noncases, consisted of a higher proportion of men, individuals with hypertension, persons with diabetes, and current smokers; a lower proportion of individuals with high education; higher mean values of age, LDL-C, and fibrinogen; and lower mean values of high-density lipoprotein cholesterol. Table 1 also shows a comparison of those with positive and negative CMV antibodies, weighted to reflect the entire ARIC Study population. Participants with positive antibody levels of CMV are less likely to be men, more likely to be African Americans, and less likely to have high education. The usual risk factors for CHD (ie, hypertension, diabetes, cigarette smoking, lipid levels, and fibrinogen levels) have small or negligible associations with CMV antibody status. Similar relationships are seen for HSV-1 (data not shown).

The distribution of antibody levels among cases and weighted noncases, shown in Table 2, is based on arbitrary intervals of the P/N ratio. This arbitrary categorization results in approximately 20% of the sample in the upper category of P/N of 6.0 or greater. The data in Table 2 are not adjusted for age or sex; thus, odds ratios are not derived from these tables.

Using the proportional hazards model, RRs of CHD by level of CMV antibodies are shown in Table 3. Two models are shown: model A adjusts for age, race, and sex using all available data and model B adjusts for age, race, sex, and important potential CHD risk factors and founders. The RRs by CMV antibody level are elevated and significant at the highest level, P/N ratio of 6.0 or greater. The RR is 1.76 when adjusting for age, race, and sex and is 1.89 when adjusting for the usual risk factors for CHD. Although the covariate effects are not displayed in Table 3, these covariates showed the strong and
CMV antibodies represents the counts of the participant’s serum to the viral antigen and to the control antigen. Titration of specific serum showed that the P/N ratios were directly related to antibody concentration, although there is not an exact translation of P/N ratios to titrated levels. For other statistical purposes, we wanted to analyze the data by increasing concentrations; thus, we arbitrarily categorized P/N ratios into 4 categories (0-1.9, 2.0-3.9, 4.0-5.9, ≥6.0). For CMV and HSV-1, this resulted in approximately 20% of the population in the highest category of 6.0 or more. The participants’ samples were stored in vials frozen at −70°C, but they had been previously thawed on 2 occasions. The thawing is not likely to affect antibody concentrations, but if there was any effect at all it would be applied equally to CHD cases and controls. The positive and negative control serum samples used in the laboratory were also repeatedly frozen and thawed, and the variation is similar to the expected variation in each test. Masked replicates of serum samples were submitted to the laboratory to evaluate laboratory repeatability. For the 35 pairs of masked replicates, there was complete agreement on positive antibody status for CMV and 91% agreement for HSV-1 (κ = 0.72).

A history of past and current cigarette smoking was obtained and, for this analysis, categorized as current smoker, former smoker, or never smoker. Fasting plasma levels of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald formula after determination of total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels. Years of education were determined and classified as less than high school graduate (low), equal to high school or vocational school (middle), or any college (high). Diabetes was classified, using definitions in effect at baseline, as a fasting blood glucose level of 7.8 mmol/L or more (≥140 mg/dL); a nonfasting blood glucose level of 11.1 mmol/L or more (≥200 mg/dL); or a history of, or treatment for, diabetes. A participant was classified as hypertensive if the average of 2 blood pressure readings was 160 mm Hg or more systolic or 95 mm Hg or more diastolic or if the participant was currently using antihypertensive medications. Fibrinogen level was determined in plasma using the thrombin time titration method.

expected associations with CHD for the usual risk factors of hypertension, diabetes, cigarette smoking, and cholesterol. Similar analyses for HSV-1 are shown in Table 3; however, there is no association between antibodies to HSV-1 and incident CHD.

In Table 4, a test of the interaction shows that for participants without diabetes, the RR of CHD for a CMV P/N ratio of 6.0 or more adjusted for race, sex, and age is 1.49 (relative to a CMV P/N ratio <6.0), but for those with diabetes, the RR is 9.15. The difference is statistically significant at P = .05, although caution should be used interpreting this effect because the sample size in the diabetic subset is small and the estimate of the RR within the diabetic group has a large variance. The data in this study show no increase in the mean values of fibrinogen in participants with positive CMV antibodies (Table 2). The introduction of fibrinogen into the covariate adjusted model (Table 3) does not diminish the CMV RRs, and there was no evidence of interaction between CMV infection and fibrinogen. A test of the interaction with fibrinogen is shown in Table 4, where no interactive effect is seen. Table 4 shows that at high levels of LDL-C, the CMV relationship with CHD is stronger but not statistically significant.

The epidemiological evidence that CMV plays a role in the atherosclerotic process in humans is derived primarily from case-control studies of atherosclerosis or of restenosis after angioplasty or transplant stenosis. In this population-based study of infection and incident CHD, we found a positive relationship between high levels of CMV antibody and CHD after accounting for other risk factors.

Several hypotheses regarding interactive effects have been raised in previous studies. In a study of patients with diabetes, Visseren et al found a relationship between CMV antibody levels and atherosclerosis and postulated that the impaired immune response to viral anti-
gens in patients with diabetes might enhance the effect of CMV on the atherosclerotic process. In the ARIC Study, we found a strong and statistically significant interaction showing that in participants with diabetes, incident CHD was 9 times higher (although with very wide confidence intervals) in persons with high CMV antibody levels compared with all others. Of related importance is the finding that shows increased atherosclerosis in immunosuppressed heart transplant recipients infected with CMV.1 However, a recent study16 of another infectious agent hypothesized to be related to CHD (Chlamydia pneumoniae) did not find a significant relationship in patients with diabetes but did in those without diabetes.

In earlier ARIC Study data4 it was hypothesized that there was an interaction of CMV with hypercoagulability and lipids. In the present data, there was no interaction with fibrinogen but there was a suggestion, although not statistically significant, that there was an interaction of CMV with hypercoagulability and lipids. In the present data, there was no interaction with fibrinogen but there was a suggestion, although not statistically significant, that there was an interaction of CMV with hypercoagulability and lipids.
philly Prospective Heart Disease Study, a nested case-control study of patients undergoing carotid endarterectomy, 36% of atherosclerotic plaques showed evidence of CMV particles compared with 0% found in normal carotid artery tissue. Atherosclerotic plaques with thrombosis showed evidence of CMV in 58% of specimens, whereas plaques without thrombosis showed evidence in only 17% of specimens. There was no association between serum antibody to CMV and the presence of CMV in the plaques. In a different study of 65 patients with CHD and 65 controls, patients had a higher prevalence of high-titer CMV antibodies compared with controls.

A much larger study of nearly 900 consecutive patients undergoing coronary angiography showed no association between CMV antibody and greater than 50% blockage in any coronary artery. However, in that study, the prevalence of positive antibodies in the control group was high (81%). Because the control group in this study included people with a clinical indication for angiography, many probably had some atherosclerosis, although they did not have significant narrowing in any one single vessel. The high prevalence in the control group makes it difficult to find a significantly elevated prevalence in the case group, but it is not incompatible with the hypothesis of involvement of CMV in earlier phases of atherogenesis.

A nested case-control study from the Physicians’ Health Study showed no relationship between CMV antibody and 50% blockage in any coronary artery. In a German study of patients with coronary stenosis (>50%) compared with blood donor controls, a nonsignificant odds ratio of 1.21 was found between IgG antibodies against CMV and cases. From a case-control series selected from general practices in the United Kingdom, an adjusted odds ratio of 1.40 (not statistically significant) was found between seropositivity to CMV (IgG) and case status. In the Caerphilly Prospective Heart Disease Study, a nested case-control study showed no association between seropositivity to CMV (IgG) and case status. In the Caerphilly Prospective Heart Disease Study, a nested case-control study showed no association between seropositivity to CMV (IgG) and case status but did show that those who were seropositive with the highest optical density had a nonsignificant odds ratio of 1.4 compared with seronegative individuals. Although these studies show either modest (nonsignificant) or no association between CMV antibodies and CHD, misclassification of case status and seropositivity can underestimate the magnitude of the relationship, and few studies examined the effect at high antibody levels.

The observation from the ARIC Study that the association between CMV antibody titers and CHD is restricted to high titer levels requires further confirmation. The elevated risk seen with the high levels of CMV antibody was also seen in earlier ARIC Study data for associations with carotid intimal-medial wall thickness and a different study of patients requiring vascular surgery. Although one could speculate that the higher antibody levels might indicate a more recent infection or reactivation of infection, the actual events that would produce the higher measured levels is not known. Under the assumption that a P/N ratio of 2.05 indeed correctly separates patients who were previously infected with CMV and those who were not, it seems that only a subset of CMV infections increase the risk of incident CHD. Previously infected patients with levels ranging from 2.0 to 5.9 had average risk ratios, after multivariable adjustment, that were smaller than the null value and not statistically significant (Table 3). Only CMV infections that yielded high P/N ratios (>6.0) were associated with a nearly 2-fold risk compared with uninfected subjects. Although one could speculate that antibody levels at the tail of the distribution indicate recent infection, reactivation of infection, or enhanced (and possibly harmful) immunologic response to CMV infection, there is little empirical data to support these theories.

A strength of this study is that the design permits estimation of the RR of incident CHD by antibody levels, although there are several issues that may affect interpretation of results. Follow-up after baseline determination of antibody levels is relatively short, a maximum of 5 years; thus, exposure to CMV early in the atherosclerotic process is not known. A causal relationship has not been established between CMV and CHD because the association could be due to opportunistic infection coincident with cofactors affecting atherosclerosis and CHD, although there is strong biological plausibility for this relationship. Adjustment for confounding may be inadequate, either because important and unknown confounders are missed or because of residual confounding. The serum sample had been stored and thawed previously, but if a bias existed because of thawing, it would affect cases and controls equally.

In summary, high levels of antibody to CMV are significantly associated with incident CHD, and this association is not due to the association of CMV with other CHD risk factors. The risk is particularly elevated in patients with diabetes, suggesting that in persons with impaired immune response, the consequences of CMV are more severe.

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