Prior Infection with Cytomegalovirus Is Not a Major Risk Factor for Angiographically Demonstrated Coronary Artery Atherosclerosis

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To determine if cytomegalovirus (CMV) infection is a risk factor for primary coronary artery disease (CAD), the association between CMV infection and CAD (≥50% blockage in any coronary artery) was investigated in nearly 900 successive nontransplant patients undergoing coronary angiography. By use of logistic regression, older age (P < .001), white race (P < .001), gender (P < .001), hypercholesterolemia (P = .04), and other established cardiovascular risk factors (P = .003) were identified as significantly associated with CAD, but CMV seropositivity (P = .462), the level of IgG antibodies to CMV whole cell antigen (P = .98), or the levels of IgG antibodies to CMV glycoprotein B (P = .67) were not. These data suggest that CMV infection is not a major risk factor for the development of primary CAD in adults.

Cytomegalovirus (CMV) plays a role in the development of certain types of coronary atherosclerosis. Following cardiac transplantation, patients with CMV infections are at significantly greater risk of developing accelerated posttransplantation coronary atherosclerosis than are patients uninfected with CMV [1]. Recently, an epidemiologic study suggests that a prior CMV infection is a risk factor for restenosis of coronary arteries following coronary atherectomy [2]. Although the exact mechanism by which CMV promotes atherosclerotic coronary occlusion is unknown, hypotheses have been suggested [3].

Less clear, however, is the role, if any, that CMV plays in the development of primary atherosclerotic heart disease. Epidemiologic studies of CMV and primary atherosclerosis are difficult because CMV is ubiquitous among humans, with ~50% becoming infected by early adult life; by late adult life (>60 years of age), nearly 90% of persons have had a CMV infection [4]. One case-control study of patients who underwent coronary bypass surgery and appropriate controls identified CMV as a risk factor for the development of primary coronary atherosclerosis [5]. Other seroepidemiologic studies of coronary atherosclerosis using different study designs have not been reported.

To determine if CMV is a risk factor for primary atherosclerotic disease in adults, we studied the association between the rate of CMV seropositivity, the titer of antibodies to CMV antigens AD169 and glycoprotein B (the major CMV envelope glycoprotein with the majority of neutralizing epitopes against CMV), and the presence or absence of coronary artery disease in nearly 900 successive patients undergoing coronary angiography.

Materials and Methods

Subjects. The subjects were all adult patients undergoing coronary angiography at the Medical College of Virginia hospitals between November 1991 and June 1992. From each patient, blood was drawn by venipuncture and collected into a 10-mL nonheparinized vacuum tube and allowed to clot at room temperature. The serum samples were centrifuged, and sera were removed, aliquoted, and stored frozen at −70°C until being assayed. Also collected was a copy of the medical data sheet for each patient, which listed a variety of demographic and health data and catheterization data.

Serologic assays. Serum IgG antibodies to CMV were first measured quantitatively with an EIA using AD169 as previously described [6, 7]. Calibrator sera and positive and negative control sera were included on each plate. All sera were diluted 1:20. Absorbance was measured on a dual wavelength Vma MicroELISA Auto Reader (Molecular Devices, Palo Alto, CA). Absorbance values (test absorbance minus control absorbance) of the calibrator sera were plotted against a standard number of antibody units determined by end-point dilution. The number of antibody units for the test serum was calculated from the regression equation; >50 antibody units was considered positive. The correlation coefficient of the calibrator sera was >.95 in all tests.

IgG antibodies to purified CMV glycoprotein B were assayed as previously described [8]. Briefly, to measure IgG antibodies to gB in sera, sera were diluted to 1:3200 and 1:6400 in duplicate in PBS-Tris (PBS-T) with 3% bovine serum albumin (BSA); 100 μL of each dilution was added per microwell precoated with 10 μg/mL of purified gB (Chiron, Emeryville, CA) in carbonate buffer (pH 9.6) or 100 μL of control antigen (10 μg/mL BSA). After 90 min at 37°C, the microwells were washed four times with PBS-T, and then 100 μL of a 1:500 dilution of alkaline phosphatase–conjugated goat anti-human immunoglobulin G (Tago, Burlin-
game, CA) in PBS-T with 3% BSA was added to each microwell. After 90 min at 37°C, the microwells were washed four times with PBS-T and then 100 μL of p-nitrophenyl phosphate (Sigma, St. Louis), 1 mg/mL in 10% diethanolamine (pH 9.6) was added to each well. The plates were incubated at room temperature until an optical density of 0.200 at 405 nm had developed with a 1:6400 dilution of a CMV-positive serum (30–60 min). The difference in absorption at 405 nm/490 nm between the control wells (coated with BSA) and the wells coated with gB was measured on a dual wavelength Vmax MicroELISA Auto Reader (Molecular Devices). All assays were run in duplicate. A standard curve for the association between optical density units and serial dilutions of a pooled intravenous immunoglobulin (IVIG) preparation (Sandoz, East Hanover, NJ) was established as shown in figure 1. For each subject, the number of IVIG antibody units per 1:3200 dilution of serum was calculated with an antibody unit defined as the natural log of the reciprocal of the dilution of IVIG required to produce the observed optical density units.

**Statistical analysis.** Potential risk factors were screened for their relationship to coronary artery disease using χ² tests or Student’s t test for univariate analysis and logistic regression for multivariate analysis. The following factors were evaluated: subject’s age, race (white or black), body mass index (defined as the weight in kilograms divided by the height in meters), sex, the presence or absence of a contributing factor (impair renal function, hypertension, congestive heart failure, or diabetes), the presence or absence of antibodies to CMV in the serum, and for seropositive subjects, the level of IgG antibodies to CMV. These factors were included in a logistic regression model for seropositivity. Data were analyzed using SAS statistical software (SAS Institute, Cary, NC), and odds ratios (OR) were calculated from the logistic regression model. P values shown in table 1 and figure 1 are for the final regression model.

**Results**

The study population comprised consecutive subjects 19–94 years old who underwent cardiac catheterization for suspected coronary artery occlusion. Twenty-eight subjects were excluded from the data analysis because they had had a previous heart transplant. Subjects were considered to have coronary artery disease (CAD) if there was a blockage in any coronary artery of >50% of the luminal diameter or a previous occlusion of >50% of any coronary artery as defined by previous coronary artery bypass grafts or previous percutaneous transluminal angioplasty. Table 1 shows the variables that were investigated as predictors of CAD.

**Association of CMV seropositivity with CAD for all subjects.** CMV seropositivity was not associated with CAD. In the final logistic regression model, age (OR = 1.05/year of increasing age), cholesterol (OR = 1.004/mg% of rising cholesterol), and race (OR = 2.08) were independently associated with an increase risk for CAD. Sex and the presence of a contributing factor were also significantly associated with CAD; however, examination of pairwise interactions revealed these had a significant interaction: Men with a contributing factor were 1.26 times more likely to have CAD than men without a contributing factor, and women with a contributing factor were 3.65 times more likely to have CAD than women without a contributing factor.

We also considered only subjects with a new diagnosis of CAD by excluding those who had had previous coronary artery bypass grafts or percutaneous transluminal angioplasties. Of 287 subjects with CAD, 245 (85.4%) were CMV-seropositive. This rate did not differ significantly from the 82% rate for 210 subjects who lacked blockage in any coronary artery or from the 81% rate for 68 subjects who had blockage of ≤50% in any coronary artery. Further, for those with any coronary artery blockage, the average reduction in luminal diameter was 77%, both for 300 subjects who were CMV-seropositive and for 55 subjects who were seronegative; for both groups, the average number of diseased vessels per subject was 1.9.

For the youngest age group, subjects <40 years of age, CMV seropositivity was not associated with CAD. Of 32 subjects <40 years of age with CAD, 72% (23) were CMV-seropositive; of 38 subjects without CAD, 76% (29) were seropositive (P > .1).

**Association of antibody levels with CAD.** We next examined the levels of IgG antibodies to AD169 antigen extracted from
Table 1. Factors associated with coronary artery disease (CAD).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects</th>
<th>All seropositive subjects age 40–70 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With CAD</td>
<td>Without CAD</td>
</tr>
<tr>
<td></td>
<td>Mean age in years ± SD (n)</td>
<td>60.8 ± 11.3 (602)</td>
</tr>
<tr>
<td></td>
<td>Mean BMI, years (n)</td>
<td>27.5 ± 5.5 (569)</td>
</tr>
<tr>
<td></td>
<td>Mean cholesterol, mg% (n)</td>
<td>210 ± 57 (520)</td>
</tr>
<tr>
<td>No. black/total no. (%)</td>
<td>200/572 (35)</td>
<td>135/222 (71)</td>
</tr>
<tr>
<td>No. with a risk factor/total no. (%)</td>
<td>414/513 (81)</td>
<td>158/222 (71)</td>
</tr>
<tr>
<td>No. women/total no. (%)</td>
<td>239/599 (40)</td>
<td>138/262 (53)</td>
</tr>
<tr>
<td>No. CMV-positive/total no. (%)</td>
<td>509/608 (84)</td>
<td>231/284 (81)</td>
</tr>
<tr>
<td>Mean no. of antibody units to CMV AD169 ± SD (n)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean no. of antibody units to CMV gB ± SD (n)</td>
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NOTE: Not all characteristics were available for each subject.

whole cells and to CMV glycoprotein B in seropositive subjects 40–70 years old. The mean number of antibody units per subject to either antigen was not associated with the presence or absence of CAD (table 1). Univariate analysis for this subject group found age, body mass index, cholesterol, race, contributing factors, and sex significantly associated with CAD; however, after consideration of all pairs of interactions in a regression model, only age \((P = .001)\), cholesterol \((P = .013)\), race \((P < .007)\), and sex \((P < .002)\) were important predictors of CAD.

For younger and older seropositive subjects, the mean number of antibody units to CMV AD169 antigen per subject was not associated with the presence or absence of CAD. For those >70 years of age, the mean number of antibody units per subject for 123 subjects with CAD was 392 (SD ± 138), not significantly different \((P = .24)\) from the mean of 354 (SD ± 132) for 22 subjects without CAD. For those <40 years of age, the mean number of antibody units per subject for 18 subjects with CAD was 311 (SD ± 116), not significantly different \((P = .075)\) from the mean of 391 (SD ± 158) for 25 subjects without CAD.

Discussion

Although over the last decade many studies have unequivocally linked CMV to accelerated posttransplantation atherosclerotic CAD among heart transplant recipients, the importance of CMV and atherosclerotic disease among immunocompetent persons has been less clear [9]. This is despite the fact that a variety of molecular techniques have revealed latent CMV in the arterial walls of both heart transplant recipients and non–heart transplant subjects with atherosclerotic heart disease [10, 11]. The importance of CMV in atherosclerosis has been given renewed attention by the recent reports that among immunocompetent patients, chronic inflammation may play a role in the development of CAD and that CMV infection may be a risk factor for restenosis following coronary arteryectomy [3, 12].

Our data from a large cohort of nontransplant patients undergoing cardiac catheterization suggests that CMV is unlikely to be an important risk factor for primary atherosclerotic heart disease. Our seroepidemiologic study is the second to investigate the association between CMV infection and CAD, and our results differ from those of the previous study performed over a decade ago by Adam et al. [5]. These authors compared the rate of CMV seropositivity and antibody titers in a group of patients with coronary atherosclerosis undergoing coronary artery bypass surgery to a matched control group of subjects with hypercholesterolemia but no history of CAD over a multiple-year period. They found that subjects had a 90% antibody prevalence rate, which was significantly higher than the 74% rate for controls, and they observed significantly higher titers to CMV antibody among subjects than controls, using CMV AD169 as the test antigen.

Our study differs from that of Adam et al. in three potentially important aspects. First, we studied nearly 900 subjects; Adam et al. included 157 subjects and an equal number of controls. Second, we used two quantitative assays: One used extracts of whole cells infected with AD169, and the other used purified CMV gB protein. This highly purified CMV gB antigen gives a low background in a quantitative EIA [6]. There is also a linear relationship between the amount of serum antibody to CMV gB and the amount of neutralizing activity in serum [8, 13]. Hence we reasoned that if patients with CAD had active ongoing CMV replication, this might be reflected in the levels of serum antibodies to gB in these patients. Third, our control population comprised patients with normal coronary arteries, and we used logistic regression to adjust for the effects of the well-known risk factors for CAD. In a study of the association of serum antibodies to *Chlamydia pneumoniae* and CAD, selection of control subjects was important [14]. When patients without angiographically demonstrated CAD were used as controls, only a weak, nonsignificant association was found between CAD and *C. pneumoniae* infection. When, however,
matched subjects without a history of CAD but who did not undergo angiography were used as controls, a significant association was observed between \textit{C. pneumoniae} infection and CAD [14].

Thus, one explanation for the difference between our results and those of Adam et al. [5] may be the selection of control subjects. A strength of our study is the large number of patients and the fact that we were able to show a significant association between CAD and the well-established cardiovascular risk factors for CAD, including high cholesterol, age, race, sex, and other contributing factors. This demonstrates that the approach we used, multiple logistic regression with controls with normal angiography, is appropriate. A potential limitation to using a series of subjects undergoing angiography is that controls may not all be disease-free. We define controls as subjects with <51% stenosis, but the majority of our control subjects (74%) had normal angiography (no stenosis in any vessel), and the rate of CMV infection among disease-free subjects was similar to that for subjects with any degree of stenosis. Another potential limitation to using a series of subjects undergoing angiography is that the persons with both CAD and specific risk factors may be selected for angiography, which may result in biased associations between potential risk factors and CAD. This, however, is unlikely to have affected our study because CMV testing was done after angiography and could not have affected subject selection. One approach to resolving the differences between our data and those of Adam et al. [5] would be longitudinal evaluation of sera from a large cohort of subjects to establish CMV infection status prior to the development of symptoms or angiographically established CAD (or both).

A potential limitation of our study was that we did not include history of cigarette smoking as a contributing factor. If we had found a significant association between CMV infection and CAD, we could not have adjusted for the effects of smoking.

After adjustment for cardiovascular risk factors, two case-control studies reported a weak nonsignificant association between CMV infection and B-mode ultrasound results as a measure of carotid artery thickening and atherosclerosis [15, 16]. One of these studies, however, did report higher antibody titers (in sera obtained at least 13 years prior to study) among cases as compared with controls [16].

If, as our data suggest, CMV is unlikely to be a significant major risk factor for the development of primary CAD, why does CMV infection appear to accelerate the rate of coronary artery occlusion following both heart transplantation and atherectomy in normal hosts? In the latter 2 cases, there is acute endothelial damage and inflammation caused either by graft-versus-host disease in the case of heart transplant recipients or by mechanical intervention in the case of restenosis. Epstein et al. have proposed that this acute endothelial damage reactivates latent CMV, the immediate-early proteins of which trigger rapid intravascular smooth muscle cell proliferation through one or more of several possible mechanisms [3]. If this hypothesis is correct, our data suggest that the endothelial damage needs to be acute rather then the chronic type associated with hypertension, hypercholesterolemia, or cigarette smoking.

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References