Cytomegalovirus in the Pathogenesis of Atherosclerosis

The Role of Inflammation as Reflected by Elevated C-Reactive Protein Levels

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OBJECTIVES We hypothesized that cytomegalovirus (CMV) infection: 1) stimulates an inflammatory response, reflected by elevated C-reactive protein (CRP) levels, and 2) predisposes to coronary artery disease (CAD), in part, through CMV-induced inflammation.

BACKGROUND Although some studies show an association between CMV and atherosclerosis, others do not. We believed that CMV exerted an atherogenic effect by inducing inflammation, and the disparate results may derive partly from individual variability in the capacity to control CMV inflammatory activity.

METHODS Blood samples were tested for CMV seropositivity and CRP levels from 238 individuals being evaluated for CAD by coronary angiography.

RESULTS An elevated CRP level (≥0.5 mg/dl) was a significant CAD determinant even after adjustment for traditional CAD risk factors (odds ratio [OR] = 2.4; p = 0.02). Moreover, CMV seropositivity was significantly associated with increased CRP levels (p = 0.04 after adjustment for CAD risk factors), suggesting that CMV could evoke a subclinical inflammatory response. However, considerable host variation existed in this response to CMV. When adjusted for CAD risk factors, the OR for CAD were 1.3 in the subgroup with CMV seropositivity alone (p = 0.7), 2.3 in the subgroup with elevated CRP levels alone (p = 0.2), and 4.3 in the subgroup with combined CMV seropositivity and elevated CRP levels (p = 0.01).

CONCLUSIONS Our results suggest that 1) CMV elicits a subclinical inflammatory response, but only in certain individuals, and 2) individuals with an inflammatory response appear susceptible to the atherogenic effects of CMV, whereas those without appear resistant. These results may partly explain the disparate results of studies attempting to relate CMV to atherogenesis. (J Am Coll Cardiol 1999;34:1738–43) © 1999 by the American College of Cardiology

Multiple epidemiological studies have suggested that cytomegalovirus (CMV) infection plays a role in the development of atherosclerosis. The data are not entirely consistent, however; some studies show a significant association with coronary artery disease (CAD) (1–8), and others do not (9,10). Of possible relevance to this conundrum are three considerations: 1) substantial evidence now exists indicating that inflammation contributes to the atherogenic process (11–18); 2) although the stimuli responsible for triggering this inflammatory process are largely unknown, infectious agents, such as CMV, may be one class of such stimuli; 3) individuals vary in their response to infection; it is therefore possible that a similar variation occurs in response to CMV infection, with only certain individuals developing an inflammatory response, and therefore only a certain subset of CMV-infected individuals being susceptible to any atherogenic effects of the virus.

Given these considerations, we studied the possible interactions that both infection and inflammatory response have on CAD risk to gain further insight into the possible role of CMV infection in CAD. The purpose of the present investigation, therefore, was to examine the validity of the following interrelated hypotheses: 1) CMV infection stimulates an inflammatory response, as reflected by elevated levels of C-reactive protein (CRP); 2) not all individuals develop an inflammatory response to CMV infection; and 3) the likelihood of CMV infection predisposing to CAD is importantly dependent on the development of an inflammatory response.
Abbreviations and Acronyms

- CAD = coronary artery disease
- CMV = cytomegalovirus
- CRP = C-reactive protein
- OR = odds ratio

METHODS

Patient characteristics. Two hundred thirty-eight individuals were recruited for this study, which was approved by the National Heart, Lung, and Blood Institute (NHLBI) the Institutional Review Board (IRB). Each individual was admitted to the Cardiology Branch, NHLBI, National Institutes of Health, for evaluation of chest pain or possibly abnormal noninvasive tests and underwent diagnostic coronary angiography. We defined a patient as having CAD if there was any angiographic evidence of atherosclerosis, including plaquing in any segment of the epicardial coronary tree. A patient was defined as being “free” of CAD only if all coronary arteries were judged to be angiographically smooth. Blood was drawn by venipuncture from each patient. The serum was aliquoted and frozen at -80°C until analyzed. No individual without CAD was admitted into the study unless blood for testing was drawn within three years of coronary angiography. No patient admitted to the study had a myocardial infarction within the previous three months. All patients with CAD were classified as having stable CAD.

Determination of risk factors for CAD. Risk factors for CAD that were analyzed included age, gender, cigarette smoking, diabetes, hypercholesterolemia, hypertension, CRP levels and seropositive CMV status. A history of past and current cigarette smoking of each patient was obtained. A patient who had stopped smoking more than 20 years ago and who was less than 30 years of age when he or she stopped smoking was considered not to have smoking as a risk factor. A patient was considered to have diabetes if he or she was taking insulin or oral hypoglycemic agents or had previously received such treatment and was currently using dietary modification to control the condition. A patient was considered to have hypercholesterolemia if he or she had a serum cholesterol value higher than 240 mg/dl (6.2 mmol/liter) or was receiving cholesterol-lowering treatment. A patient was considered to have hypertension if he or she had received the diagnosis or was being treated with antihypertensive medications and/or dietary modification.

Antibody status to CMV. Blood samples from each individual were tested for anti-CMV IgG antibodies using an enzyme-linked immunosorbent assay (ELISA, CYTOMEGELISA II, Biowhittaker, Walkersville, Maryland). Antibody results were calculated from standard curves provided by the manufacturer. The threshold value for a “positive” result was determined prospectively: an ELISA value <0.25 units was considered a negative result, and a value of 0.25 units was considered positive, indicating prior exposure to CMV. Samples for anti-CMV IgG antibodies were tested in triplicate and in two separate experiments.

C-reactive protein. Serum CRP was measured by fluorescence polarization immunoassay (FPIA) technology (TDxFLEx analyzer, Abbott, Abbott Park, Illinois). Using this assay, 95% and 98% of healthy individuals (n = 202) had a CRP level of ≤0.5 mg/dl and ≤1.0 mg/dl, respectively, in their sera (19). The between-run coefficient of variation (CV) of this assay (n = 31) was 4.3% and 2.2% at mean levels of 1.10 mg/dl and 2.94 mg/dl, respectively.

Statistical analysis. Categorical data were analyzed by the chi-square test or the Fisher exact test for small samples. All tests were two-sided. The dichotomous variable indicating presence or absence of CAD was modeled as a function of other factors or variables using multiple logistic regression. The odds ratio (OR) was used as a measure of the risk of CAD in patients with a given risk factor as compared to those without that factor. In the case where age was the risk factor, the OR measures the change in risk of CAD for a ten-year increase in age. The covariates considered were age, gender, cigarette smoking, diabetes, hypercholesterolemia, hypertension, and seropositive CMV status. All covariates were examined as predictors of both CRP level and the presence of CAD in univariate analyses using the Pearson correlation. A multivariate analysis of these covariates as predictors of CAD was made using the Logistic procedure of the SAS software system for PC Windows (20), which computes the logistic regression of the dichotomous variable CAD on age, gender, and other factors. The relationship between CRP level, a continuous variable, and the values of age, gender, and so forth, was analyzed using the General Linear Model (GLM) procedure of SAS.

RESULTS

Association of CAD with traditional risk factors. Of the 238 study subjects, 151 (63%) were men and 87 (37%) women. One hundred sixty-nine (71%) were white, 34 (14%) were black, 22 (9%) were Asian, and 13 (5%) were unknown. Their ages ranged from 30 to 81 years (mean 57.2 years, median 57 years). There were 158 (66%) individuals who had evidence of CAD ranging from plaquing to significant stenoses by coronary angiography. By univariate analysis, the factors that were significantly associated with the presence of CAD were age (p = 0.0001), male gender (p = 0.0002), smoking (p = 0.008), diabetes (p = 0.001) and hypercholesterolemia (p = 0.0004). Age, male gender and hypercholesterolemia were retained as significant risk factors after multivariate analysis (Table 1). Thus, our study cohort was representative of a typical population of patients with stable CAD.
Association of elevated CRP levels with CAD risk. Elevated levels of CRP were found to be an important risk factor for CAD, as shown in Figure 1. Mean CRP value was significantly higher in patients with CAD, compared to those without CAD (0.89 ± 0.05 vs. 0.68 ± 0.07 mg/dl, p = 0.01, Fig. 1A). Seventy-seven percent of patients with CAD had CRP levels >0.5 mg/dl, versus 52% of patients without CAD (0.05 = 0.001, Fig 1B). Elevated CRP level remained a significant determinant of CAD after adjustment for traditional CAD risk factors, including age, male gender, smoking, diabetes, hypertension and hypercholesterolemia (OR = 2.4, p = 0.02).

Contribution of CMV infection to elevated CRP levels. Of the 238 patients, 153 (64%) had positive tests for anti-CMV IgG antibodies. Mean value of CRP was 0.88 ± 0.05 mg/dl in CMV seropositive patients and 0.69 ± 0.06 mg/dl in CMV seronegative patients (p = 0.02). By univariate analysis, the CRP level (as a continuous variable) correlated with presence of hypercholesterolemia (p = 0.006), hypertension (p = 0.0008), and CMV seropositivity (p = 0.02), but not with age, male gender, smoking and diabetes (all p >0.1). When adjusted for traditional CAD risk factors, including age, male gender, smoking, diabetes, hypertension and hypercholesterolemia, by multivariate analysis an association between CRP level and CMV seropositivity retained statistical significance (p = 0.04, Table 2), suggesting that CMV infection is an independent determinant of CRP levels.

Association between CMV seropositivity and CAD risk. On univariate analysis, CMV seropositivity was significantly associated with CAD (p = 0.02). Of 158 patients with CAD, 110 (70%) had anti-CMV IgG antibodies, whereas

Table 1. Association of Traditional Risk Factors With Presence of CAD

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Patients With CAD (%)</th>
<th>Patients Without CAD (%)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency of Factors</td>
<td></td>
<td>Pearson Correlation</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td></td>
<td>Patients With CAD</td>
<td>Patients Without CAD</td>
<td>p Value</td>
<td>p Value</td>
</tr>
<tr>
<td>Age*</td>
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<td>Male gender</td>
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<td>Smoking</td>
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<td>0.1737</td>
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<td>Diabetes</td>
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<td>0.0010</td>
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<td>Cholesterol</td>
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<td>0.2284</td>
<td>0.0004</td>
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<td>Hypertension</td>
<td>52</td>
<td>32</td>
<td>0.0911</td>
<td>0.1669</td>
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</tbody>
</table>

*Age as a continuous variable. †Odds ratio for age represents the increase in risk of CAD for each 10-year increase in age.
only 43 of 80 (54%) of those with no evidence of CAD were CMV seropositive \( (p = 0.02, \text{Fig. 2}) \). In addition, we carried out multiple logistic regression analysis to assess determinants of CAD; covariants included traditional risk factors, elevated CRP levels \( (>0.5 \text{ mg/dl}) \) and CMV seropositivity. We found that elevated CRP levels retained a significant association with CAD \( (\text{OR} = 2.3, p = 0.02) \), whereas the association of CMV seropositivity with CAD lost significance \( (p = 0.2) \).

**Variation in the inflammatory response to CMV infection.** Further analysis showed considerable variation in the host's inflammatory response (judged by elevated CRP levels) to CMV infection: 111 of 153 (73%) of CMV-seropositive individuals had elevated CRP levels \( (>0.5 \text{ mg/dl}) \), whereas the remainder of the seropositive individuals (27%) had CRP values \( <0.5 \text{ mg/dl} \). Figure 3 shows the influence of this variability on the relation between CMV seropositivity and CAD prevalence. There is a stepwise increase in the prevalence of CAD depending on CMV seropositivity and/or elevated CRP levels. The highest CAD prevalence occurred in the subgroup with combined seropositivity and elevated CRP levels \( (83/107, 78\%) \). However, only 22% \( (24/107) \) of individuals without CAD had both CMV seropositivity and elevated CRP levels. When adjusted for CAD risk factors, the ORs for CAD were 1.3 \( (95\% \text{ CI}, 0.4 \text{ to } 4.4; p = 0.7) \) in the subgroup with CMV seropositivity alone, 2.3 \( (95\% \text{ CI}, 0.7 \text{ to } 7.5; p = 0.2) \) in the subgroup with elevated CRP levels alone, and 4.3 \( (95\% \text{ CI}, 1.4 \text{ to } 13.1; p = 0.01) \) in the subgroup with combined CMV seropositivity and elevated CRP levels. Overall linear trend achieves significance even after adjustment for CAD risk factors \( (p = 0.0016) \). CMV Ab+ or CMV Ab−: CMV antibody response positive or negative, respectively; CRP >0.5 or CRP ≤0.5: CRP levels >0.5 mg/dl or CRP levels ≤0.5 mg/dl, respectively.

**DISCUSSION**

The hypotheses we prospectively tested in this investigation were designed to provide insight into both the mechanisms by which infection, and in particular CMV, might contribute to CAD, and why the results of multiple epidemiological studies assessing the potential role of CMV in CAD have not been entirely consistent \( (1\text{–}10) \). These hypotheses...
were predicated on the concepts that CMV stimulates an inflammatory response in the host, that such a response varies among hosts, and that the presence or absence of an inflammatory response will importantly influence risk of CAD prevalence. Our results are consistent with these hypotheses.

Relation among CRP levels, CMV infection, and CAD. We demonstrated an association between antibodies to CMV and elevated CRP levels, as reflected by the observation that higher CRP levels were present in CMV-seropositive versus CMV-seronegative individuals (0.88 versus 0.69 mg/dl). This difference retained significance even on multivariate analysis after adjustment for traditional CAD risk factors, including age, male gender, smoking, diabetes and hypercholesterolemia (p = 0.04), suggesting that CMV infection is an independent determinant of elevated CRP levels and, by inference, predisposes to the induction of chronic subclinical inflammation in the host. Importantly, however, we also found that not all hosts respond in this way; of the CMV-seropositive individuals, 27% did not have elevated levels of CRP.

This variation in the inflammatory response of the host to CMV infection likely has important biologic consequences, for in our total cohort, although CMV infection was not an independent determinant of CAD risk, CRP elevation was. Consistent with the concept that the presence or absence of an inflammatory response to CMV infection importantly influences CAD risk is the finding of a stepwise increase in the prevalence of CAD depending on a positive interaction between CMV seropositivity and elevated CRP levels (Fig. 3). The highest CAD prevalence (78%) occurred in the subgroup with combined seropositivity and elevated CRP levels, providing an OR for CAD of 4.3 (p = 0.01), compared to an OR of 1.3 in the subgroup with CMV seropositivity alone (p = 0.7), and 2.3 in the subgroup with elevated CRP levels alone (p = 0.2). The overall stepwise increase in CAD prevalence remained significant by multiple logistic regression analysis (p = 0.0016). Thus, one mechanism by which CMV may contribute to atherogenesis is through provocation of an inflammatory response in the host.

Our results also provide a potential explanation for the conflicting evidence relating to the role of CMV in atherosclerosis, as it appears that the particular nature of the host’s inflammatory response is one important determinant of whether CMV contributes to CAD. This conclusion implies that studies assessing whether CMV infection (or probably any infectious agent) is associated with CAD must take into consideration the frequency with which infection leads to inflammation in the particular cohort being studied. It could be anticipated that on the basis of genetic, environmental, or selection factors, different populations being studied may exhibit different dominant modes of responding to a specific infection—the dominant response of one population may be to develop an inflammatory response, whereas such a response may be absent in another population. Whether or not an inflammatory response evolves may in turn depend on the type of immune response that develops—immunodominant humoral or immunodominant cellular.

Prior studies of CRP and CMV infection in CAD. These considerations may relate to the apparently discordant results between our study, which demonstrated a significant association between CMV seropositivity and elevated CRP levels, and the lack of such a significant association in the study published by Anderson et al. (21), who reported on the relation between seropositivity to three pathogens—CMV, Chlamydia pneumoniae, and Helicobacter pylori—and elevated CRP levels. They found, concordant with the results of many other studies, that CRP levels were significantly higher in individuals with CAD versus those without CAD. They also reported no significant correlation between CAD prevalence and seropositivity to any of the pathogens, a result compatible with our findings. However, in contrast to our results, Anderson et al. (21) could find no significant association between elevated CRP levels and any of the pathogens.

The disparate results between our study and that of Anderson et al. (21) may also relate to the type of patient included in each of the studies. In contrast to the inclusion criteria for the individuals entered into the present study, in which patients with recent myocardial infarction were excluded, Anderson et al. (21) did admit study patients with recent infarction. Myocardial infarction by itself can cause substantial increases in CRP levels. It is therefore possible that the presence of this independent factor leading to increased CRP levels exerted confounding influences accounting for the failure to establish a significant association between CMV seropositivity and elevated CRP levels. In addition, there were differences in definition of CAD versus no CAD between the two studies. Anderson et al. (21) entered patients into the CAD group if they had >60% stenoses, and into the no-CAD group if they had <10% stenoses. Individuals with intermediate stenoses (10% to 60%) were excluded from the study. In contrast, we defined an individual as having CAD if there was any luminal wall irregularity, and as being free of CAD only in the presence of absolutely smooth coronary arteries on angiography.

Study limitations. The conclusions of the present investigation must be placed in the context of several caveats. First, the study design of this investigation is cross-sectional in nature. Such a design cannot establish causality. It can only establish an association. Any conclusion derived from such a study must therefore be considered preliminary and hypothesis-generating rather than hypothesis-proving. Second, as implied in our discussion about different populations responding differently to a specific infection, the conclusion that CMV contributes to CAD only insofar as it contributes to inflammation may not be true for all individuals with CMV infection and CAD. It is possible, for example, that certain individuals who do not develop an inflammatory response to CMV infection develop a type of immune response that might itself contribute to atherogenesis. Third, not all individuals...
who have elevated CRP levels and are seropositive for CMV have associated CAD. This is consistent with the concept that because CAD is a multifactorial disease, any one risk factor will rarely be sufficient for disease development.

Finally, one of the strengths of our study is that our control and patient groups were defined by coronary angiography, a far more accurate means of defining presence or absence of CAD than the clinical parameters employed in most seroepidemiologic studies. However, we cannot exclude the possibility that some individuals considered to be free of CAD had minimal disease that could not be detected by angiography. In addition, our non-CAD control group consisted of individuals who, on clinical evaluation, had some suspicion of CAD. The absence of angiographically evident CAD is definitive evidence that the findings leading to angiography were not due to CAD; however, such individuals may not be representative of other individuals without CAD who lack clinical features triggering the decision to perform coronary angiography.

Conclusions. The results of this investigation demonstrate that although CMV infection is associated with elevated CRP levels, individuals vary in their capacity to control CMV inflammatory activity, as reflected by elevated CRP levels. This variation appears to be biologically relevant, as the risk of CAD is greatest in individuals who are seropositive for CMV and who have elevated CRP levels. These results are compatible with the hypothesis that susceptibility to the atherogenic effects of CMV depends, at least in part, on the capacity of the host to suppress CMV-induced inflammatory activity. Based on these and related findings, it is likely that a complete understanding of the potential role of infection in CAD will be achieved only after the more complex interrelations between a pathogen and the host's response to the pathogen are elucidated.

Acknowledgments

We thank Rita Mincemoyer for her excellent clinical assistance and data acquisition, Bill Schenke for his kindly help in the preparation of manuscript figures, and Rene Costello for his excellent technical assistance.

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REFERENCES


