The Possible Role of Hepatitis A Virus in the Pathogenesis of Atherosclerosis

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The possible association between hepatitis A virus (HAV) infection and coronary artery disease (CAD) was studied. Blood from 391 patients undergoing coronary angiography was tested for serum IgG antibodies to HAV and C-reactive protein (CRP). Of the 391 patients, 205 (52%) had anti-HAV IgG antibodies. CAD prevalence was 74% in HAV-seropositive and 52% in HAV-seronegative patients (P < .0001); significance persisted after adjustment for either traditional CAD risk factors or for risk factors plus other infectious agents (cytomegalovirus, Chlamydia pneumoniae, Helicobacter pylori, and herpes simplex virus). In addition, CRP levels were significantly higher in HAV-seropositive than in HAV-seronegative patients (P = .013) in both univariate and multivariate analyses. Logistic regression analysis demonstrated that HAV seropositivity is an independent predictor of risk for CAD and elevated CRP levels. HAV infection is therefore associated with CAD, which raises the possibility that this virus may play a causal role in atherogenesis.

Substantial evidence now exists indicating that inflammation plays an important role in atherogenesis [1–5]. An intensive search for the stimuli that trigger and maintain the inflammatory process is underway. Of relevance to this concept are the results of epidemiologic studies suggesting possible atherogenic roles for such pathogens as cytomegalovirus (CMV), Chlamydia pneumoniae, Helicobacter pylori, and herpes simplex virus (HSV) [6–14]. Therefore, we and others have focused efforts on determining whether infectious agents are among the stimuli that trigger and maintain an inflammatory state and thereby contribute to atherosclerosis.

There is an important shared characteristic of the infectious agents implicated in the development of atherosclerosis: they all are intracellular pathogens and can establish long-term, persistent infection or induce long-lasting effects, such as persistent circulating antibodies. This fact led us to examine the hypothesis that other intracellular pathogens associated with a persistent antibody response, in addition to those already identified, contribute to atherosclerosis.

We thought that hepatitis A virus (HAV) would be a reasonable candidate pathogen to test our hypothesis. Although acute infection with this virus leads to hepatitis, it does not produce persistent liver disease, as do hepatitis B and hepatitis C viruses. HAV is commonly believed to be eliminated from the body after the acute infection, and there are no other target tissues in which this virus is known to reside or to produce disease. Anti-HAV IgG antibodies persist probably for the life of the host, with ~50% of individuals in developed countries being seropositive. However, there is currently no evidence that persistent HAV antibodies reflect persistent viral infection.

In a recently published paper relating to the role of multiple pathogens (pathogen burden) in atherosclerosis, we described, in brief, a strong association between HAV and coronary artery disease (CAD) in a cohort of 238 individuals [15]. That original cohort now has been increased to 391 individuals, and the present investigation presents an in-depth analysis of the relationship between HAV and CAD. Because inflammation is believed to be a contributing mechanism in atherosclerosis, we used elevated serum levels of C-reactive protein (CRP) as a marker of an underlying inflammatory process and determined whether prior HAV infection is involved in inducing chronic inflammation. Such a relationship would establish a mechanism by which HAV could predispose to the development of atherosclerosis.

Therefore, the goal of the present investigation was to determine, in a larger cohort, whether there is, in fact, an independent association between HAV seropositivity and CAD and whether such an association is related to chronic inflammation, as judged by elevated CRP levels.

Methods

Study subjects. Three hundred ninety-one individuals entered the study. The study cohort consisted of individuals who were referred for coronary angiography because of chest pain or non-invasive tests compatible with myocardial ischemia. We defined a patient as having CAD, if there was angiographic evidence of ath-
erosclerosis (≥50% stenosis of ≥1 major coronary artery by coronary angiography). Patients with significant valvular heart disease or nonatherosclerotic cardiomyopathy were excluded. No patient admitted to study had had a myocardial infarction within the previous 3 months.

**CAD risk factors.** Risk factors for CAD that were analyzed included age, race, male sex, cigarette smoking, diabetes, hypercholesterolemia, hypertension, elevated CRP levels, seropositive status to HAV, and occupational status. Patients were asked to classify their race as white, black, or Asian. A history of past and current cigarette smoking was obtained for each patient. Those who had stopped smoking ≥20 years ago and who were <30 years old when they stopped smoking were considered not to have smoking as a risk factor. Patients were considered to have diabetes if they were taking insulin, oral hypoglycemic agents, had previously received such treatment, or were currently using dietary modification to control the condition. Patients were considered to have hypercholesterolemia if they had a serum cholesterol value >240 mg/dL (6.2 μmol/L) or were receiving cholesterol-lowering treatment. Patients were considered to have hypertension if they had received such a diagnosis with arterial pressure >140/90 mm Hg or were being treated with antihypertensive medications or dietary modification. We used occupational status as an indicator of socioeconomic status, and categorized professionals and persons in supervisory positions as having high occupational status; the remaining subjects were assigned to low occupational status.

**Serum IgG antibodies to HAV.** Serum samples obtained from all study subjects were frozen at −80°C, and aliquots were thawed when needed for specific tests. An EIA (HAVAB; Abbott Laboratories, Abbott Park, IL) was used to determine serum IgG antibodies for HAV. The presence or absence of anti-HAV antibodies was determined by comparing the absorbance value of the sample to a cutoff value. This cutoff value was calculated from the negative and positive control absorbance values, as explained by the manufacturer. Specimens with absorbance values less than or equal to the cutoff value are considered to be negative for anti-HAV antibodies. Specimens with absorbance values greater than the cutoff value are considered to be reactive for anti-HAV antibodies.

**Serum IgG antibodies to CMV, C. pneumoniae, H. pylori, HSV-1, or HSV-2.** Commercially available ELISA kits were used to determine serum IgG antibodies to CMV (Wampole, Cranbury, NJ) and to H. pylori (Meridian Diagnostics, Cincinnati). Serum IgG antibodies to C. pneumoniae were determined in 238 patient samples by microimmunoﬂuorescence assay (Dept. of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle) and in 153 patient samples by ELISA (Savvyon Diagnostics, Ashdod, Israel). By use of the microimmunoﬂuorescence assay as the standard, the ELISA assay provided results that were 95% concordant (data not shown). Serum IgG antibodies for HSV-1 and HSV-2 were determined at American Medical Laboratories (Chantilly, VA), using an EIA.

**Detection of serum CRP.** Serum CRP was measured by ﬂuorescence polarization immunoassay (TDxFLEx analyzer; Abbott Laboratories) technology. As determined by use of this assay, 95% of healthy individuals (n = 202) had a serum CRP level <0.5 mg/dL, and 98% had <1.0 mg/dL. The between-run coefficient of variation of this assay (n = 31) was 4.3% and 2.2% at mean levels of 1.10 and 2.94 mg/dL, respectively.

**Statistical analysis.** Categorical data were analyzed by use of the χ² test or Fisher’s exact test for small samples. All tests were 2-sided. Analyses of CRP serum level in relation to HAV and other factors were made by multiple regression of the log of the CRP level on these variables. Values of r, the estimated Pearson correlation, indicate the strength of the relationships. The dichotomous variable indicating the presence or absence of CAD was modeled as a function of other factors, 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, compared with those without that factor, or as a multiplicative factor for each unit increase in age or HAV infection. The covariates considered were age, race, sex, cigarette smoking, diabetes, hypercholesterolemia, and hypertension (the traditional CAD risk factors); seropositive status to HAV, CMV, C. pneumoniae, H. pylori, HSV-1, and HSV-2; and occupational status. All covariates were examined as predictors of CRP and CAD in univariate analyses and as a group in 1 multivariate model.

**Results.** Three hundred ninety-one subjects were studied. Their ages ranged from 30 to 81 years (mean, 57.5 years; median, 58.0 years). There were 244 (62%) men, 284 (73%) whites, and 248 (63%) with angiographic evidence of CAD. Of the patients with CAD, 194 (78%) of 248 had CRP levels >0.5 mg/dL, compared with 96 (67%) of the 143 patients without CAD (P = .015). With the exceptions of smoking and hypertension, traditional CAD risk factors (age, male sex, diabetes, and hypercholesterolemia) and elevated CRP levels (>0.5 mg/dL) were significantly associated with the risk of CAD, by both univariate and multivariate analyses.

**HAV seropositivity and risk of CAD.** In our study cohort, 205 (52%) of the 391 subjects had IgG antibodies directed against HAV. The prevalence of CAD was 74% (151/205) in the HAV-seropositive patients and 52% (97/186) in the HAV-seronegative patients. The increased prevalence of CAD in patients with HAV seropositivity was significant (P < .0001), and the association between CAD and HAV seropositivity retained significance after multivariate analysis was done to adjust for the following: (1) the 7 traditional CAD risk factors (age, race, male sex, smoking, diabetes, hypercholesterolemia, and hypertension) and elevated CRP levels (P = .003; adjusted ORs are shown in figure 1) or (2) for these risk factors plus other infectious agents, such as CMV, C. pneumoniae, H. pylori, HSV-1, and HSV-2, (adjusted OR, 2.1; 95% confidence interval [CI], 1.2–3.9; P = .01).

**HAV seropositivity and risk factors.** The association between HAV seropositivity and risk factors is presented in table 1. HAV seropositivity was not associated with male sex, smoking, diabetes, or hypercholesterolemia; however, as expected, age and race were significantly associated with HAV infection. It is in-
Figure 1. Adjusted odds ratios with 95% confidence intervals (CIs) for coronary artery disease (CAD) in various risk-factor groups. Adjusted covariates include age (in 10-year increments), race (white vs. nonwhite), male sex, smoking, diabetes, hypercholesterolemia, hypertension, elevated C-reactive protein (CRP) levels, and hepatitis A virus (HAV) seropositivity.

Table 1. Association of hepatitis A virus (HAV) seropositivity with traditional coronary artery disease risk factors.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Frequency of factors, %</th>
<th>HAV-seropositive patients (n = 205)</th>
<th>HAV-seronegative patients (n = 186)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.8 ± 0.76</td>
<td>54.8 ± 0.89</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>60.5</td>
<td>86.0</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>62.9</td>
<td>61.8</td>
<td>.8227</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>49.3</td>
<td>52.7</td>
<td>.4970</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>21.9</td>
<td>19.4</td>
<td>.5270</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>52.7</td>
<td>51.1</td>
<td>.7507</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>56.6</td>
<td>45.4</td>
<td>.0274</td>
<td></td>
</tr>
</tbody>
</table>

* Univariate analysis.
* Data are mean ± SE of percentage of patients.
* White vs. nonwhite.
seropositivity and CAD in both groups are shown in figure 2. The group with low CRP values exhibited the greater effect of HAV seropositivity on adjusted OR for CAD.

Discussion

The results of the present investigation demonstrate that anti-HAV IgG antibodies are independently associated with both CAD and elevated CRP levels. Because elevated CRP levels are believed to reflect a chronic, persistent inflammatory state and are associated with an increased rate of cardiovascular events [1–5], the data presented here suggest that, if, in fact, the association we have demonstrated reflects a causal role of HAV infection in atherogenesis, one of the likely mechanisms is through stimulation of inflammatory responses.

The observations that anti-HAV antibodies are associated with CAD prevalence and with elevated CRP levels raise the possibility that HAV can establish a chronic, persistent infection that leads to chronic inflammation that has important biologic consequences. To our knowledge, no prior evidence has been published suggesting that HAV can persist in its host or establish a chronic, subclinical inflammatory condition. Although this is one possible interpretation of our observations, however, other explanations are equally plausible. For example, there can be (1) repeated subclinical infection by hepatitis A, which results in the persistence of anti-HAV antibodies and elevated CRP levels without persistent HAV infection; (2) repeated infections by other pathogens that share cross-reactive antigenic epitopes with HAV and thereby result in persistence of anti-HAV antibodies and elevated CRP levels; (3) an initial infection with hepatitis A that leads, through molecular mimicry, to an autoimmune response targeted to homologous cross-reactive host peptides, leading to an autoimmune contribution to vascular disease.

Any of these mechanisms leading to persistent anti-HAV antibodies can be responsible for the association of HAV seropositivity with CAD and with elevated CRP levels, even after clearance of HAV from the infected host and, therefore, in the absence of persistent HAV infection. Thus, the highly significant associations between HAV seropositivity and CAD and between HAV seropositivity and elevated CRP do not necessarily reflect persistent HAV infection.

An additional point that needs to be emphasized is that, although multiple logistic regression analysis demonstrated that HAV seropositivity is an independent determinant of elevated CRP levels, the increased levels of CRP could be contributed to by a number of additional factors not tested in our model. This could include an acute inflammatory process, a concomitant infection with a pathogen other than HAV and the other infectious agents tested in the model, or even to the underlying inflammatory processes involved in CAD. It is also important to point out that multiple logistic regression analysis demonstrated that HAV is a predictor of CAD, independent of elevated CRP levels. In addition, when the study group was divided into those individuals with CRP levels above and those with CRP levels below the median level for the group, CAD prevalence was significantly associated with HAV seropositivity in the low CRP group. Our results are, therefore, compatible with the hypothesis that HAV infection can contribute to CAD through the associated inflammatory response (insofar as elevated CRP levels reflect underlying inflammation) and through mechanisms independent of those related to the type of inflammation associated with elevated CRP levels. One possibility, as noted above, is that anti-HAV antibodies might cross-react with host peptides, leading to an autoimmune contribution to vascular disease.

Figure 2. Effects of hepatitis A virus (HAV) seropositivity on adjusted odds ratio (OR) for coronary artery disease (CAD) in groups with C-reactive protein (CRP) levels below or above the median value (0.82 mg/dL). Adjusted covariates include age (in 10-year increments), race (white vs. nonwhite), male sex, smoking, diabetes, hypercholesterolemia, hypertension, and HAV seropositivity. Adjusted ORs for CAD were 3.6 (95% confidence interval [CI], 1.44–9.17; P = .006) in the group with low CRP values and 1.6 (95% CI, 0.79–3.46; P = .18) in the group with high CRP values. HAV Ab(+) and HAV Ab(−), HAV seropositive and seronegative, respectively.
The data suggesting that HAV may contribute to CAD through the chronic inflammatory response it evokes and the considerations relating to an autoimmune contribution raise an interesting point. If it is granted that other infectious agents may contribute to atherosclerosis, given that HAV is biologically different from the other commonly cited “atherogenic” pathogens and presents a very different clinical picture, how does it, with its different features, contribute to the disease? Does it have a unique pathway leading to atherosclerosis? We have no definitive answer to these questions. However, we believe that the results of this and other studies suggest that there may be common mechanistic pathways that are shared by different pathogens, even though each has different biological and clinical features. These common pathways likely derive in part from the inflammatory and immune responses evoked by all pathogens.

There are several caveats relating to our study that are important to note. First, the study design was cross-sectional in nature, and 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, our non-CAD control group consisted of individuals who, on clinical evaluation, had suspected CAD. These individuals may not be representative of other individuals without CAD who lack clinical features triggering the decision to perform coronary angiography. Third, it is possible that the association between CAD and HAV is actually a reflection of a situation in which an individual with CAD becomes more susceptible to HAV infection, possibly as a result of the presence of other risk factors that are the primary contributors to atherogenesis. Fourth, there may be something unusual about our study population, because HAV exposure was found to be associated with CAD, but hypertension and smoking were not (figure 1).

Other possibilities should also be considered. It is possible that HAV is only a marker of another independent risk factor for CAD. This possibility is unlikely, given the fact that multivariate analysis using traditional risk factors (i.e., age, race, male sex, cigarette smoking, diabetes, hypercholesterolemia, hypertension, and occupational status) and pathogens (CMV, C. pneumoniae, H. pylori, HSV-1, and HSV-2) establish HAV as an independent predictor of CAD. It is also possible that, although the associations we report between HAV seropositivity, CRP levels, and CAD are significant, other factors not considered in our study may contribute to the significant associations we have identified. For example, it is possible that differences in the prevalence of HAV between persons with and those without CAD could be due to behavioral or socioeconomic factors, such as sexual orientation and travel history, that were not evaluated in this study.

In summary, our results are compatible with the hypothesis that prior HAV infection is associated with the development of CAD. The potential clinical importance of a causal role of HAV infection in atherosclerosis and the fact that the cross-sectional design of the present study does not permit definitive conclusions regarding causality should stimulate the initiation of further studies to determine whether HAV is indeed a causative factor in atherogenesis.

References