Endothelial Adhesion Molecules Are Associated with Inflammation in Subjects with HIV Disease

Mark M. Melendez, Margaret A. McNurlan, Dennis C. Mynarcik, Shilpi Khan, and Marie C. Gelato
Departments of Surgery and Medicine, Stony Brook University Medical Center, Stony Brook, New York

Background. The presence of the adhesion molecules intercellular adhesion molecule–1 (ICAM-1) and soluble vascular cell adhesion molecule–1 (VCAM-1) is associated with elevated risk of cardiovascular disease. Subjects with human immunodeficiency virus (HIV) disease have multiple risk factors for cardiovascular disease, including elevated serum lipid levels, insulin resistance, and elevated levels of ICAM-1 and VCAM-1. This study assessed the variables associated with elevated adhesion molecule levels in this patient population.

Methods. Serum levels of ICAM-1 and VCAM-1 were assessed in 31 subjects without HIV disease and 52 subjects with HIV disease. Pearson correlation indicated a significant relationship between ICAM concentration and other variables, including CD4+ cell count, HIV viral burden, insulin sensitivity, and serum lipid level. Multiple regression modeling was used to determine the strengths of association among the variables.

Results. Subjects with HIV disease had elevated levels of ICAM-1 and VCAM-1. Pearson correlation analysis revealed significant associations between ICAM-1 and VCAM-1 level and insulin sensitivity, plasma lipid level, and presence of type 2 soluble receptor for tumor necrosis factor–α (sTNFR2). With multiple regression modeling to control for interdependence, only sTNFR2, a marker of inflammation, was an independent predictor of ICAM-1 and VCAM-1 levels.

Conclusions. The study suggests that many of the variables associated with ICAM-1 and VCAM-1 levels can be related to their impact on inflammation.

Although current antiretroviral regimens have decreased the mortality and morbidity associated with HIV infection, metabolic abnormalities, including dyslipidemia, insulin resistance, and alterations in body fat distribution (i.e., increased amounts of visceral adipose tissue and/or decreased amounts of peripheral adipose tissue, known as HIV-associated lipodystrophy or lipodystrophy), now cause concern [1–4]. The etiology of this metabolic syndrome is not known, although a number of factors undoubtedly contribute.

The components of the metabolic syndrome associated with HIV infection—insulin resistance, visceral adiposity, and dyslipidemia—are all risk factors for cardiovascular disease, and increased risk of cardiovascular disease has been reported in HIV-infected patients [5].

The increase in risk of cardiovascular disease has also been confirmed by 2 studies demonstrating both an increased rate of premature coronary artery disease [6] and an increased rate of myocardial infarction [7, 8] in patients with HIV disease.

Increased risk of cardiovascular disease has also been associated with elevated circulating levels of 2 adhesion molecules released by the vascular endothelium: soluble intercellular adhesion molecule–1 (ICAM-1) and vascular adhesion molecule–1 (VCAM-1) in both apparently healthy individuals [9–11] and individuals with known coronary artery disease [12]. The circulating levels of these adhesion molecules have also been assessed in patients with HIV disease. An elevation in levels of endothelial-cell derived markers, such as von Willebrand factor antigen and soluble vascular cell adhesion molecule-1, has been reported in patients with HIV disease, particularly in patients with the highest viral burden and the most advanced disease [13–16]. More recently, de Larranaga et al. [17] demonstrated that, even when HAART results in a decreased viral burden and an increased CD4+ cell count, HIV-infected patients still have elevated levels of endothelial...
markers. The levels of these markers were also higher in patients who had HIV-associated lipodystrophy than in those who were receiving HAART who did not have HIV-associated lipodystrophy.

We previously demonstrated that 2 components of the HIV-associated metabolic syndrome—insulin resistance [18] and loss of the proportion of body fat present in the limbs [18]—were related to inflammation. The present study of 83 subjects was undertaken to determine whether an additional risk factor for cardiovascular disease—elevated VCAM-1 and ICAM-1 levels—was also related to inflammation.

SUBJECTS AND METHODS

Thirty-one individuals without HIV disease (control subjects) and 52 subjects infected with HIV participated in this study. All study subjects had been free of acute illness for the 3 months preceding their participation in the study, and HIV-infected subjects had not changed antiretroviral medications in the preceding 3 months. Subjects with overt diabetes mellitus (fasting glucose level, >126 mg/dL) were excluded from the study. The study was approved by the Committee on Research Involving Human Subjects at the University Medical Center of the State University of New York at Stony Brook, and all subjects provided written informed consent.

Study subjects were admitted to the Stony Brook General Clinical Research Center on the evening before the study. Subjects fasted overnight, following a snack at 10:00 p.m. Venous blood samples were taken the following morning between 7:00 and 8:00 a.m. for the determination of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride, and glucose levels.

Serum lipid levels were assessed in the Department of Clinical Chemistry at Stony Brook Hospital. Triglyceride levels were assessed with an automated colorimetric assay based on lipoprotein lipase oxidation (Roche Modular GPO-PAP method; Roche). The HDL cholesterol level was determined with a coupled, colorimetric cholesterol esterase and cholesterol oxidase assay (Roche Modular, HDL-Cplus, Second Generation; Roche), and LDL cholesterol level was calculated on the basis of the difference between the HDL cholesterol level and the total cholesterol level. The CD4+ cell count and viral burden were also determined by the Department of Clinical Chemistry. CD4+ cell counts were determined by flow cytometry, and viral burden was determined by PCR.

Blood samples obtained for assessment of VCAM-1, ICAM-1, and type 2 soluble receptor for TNF-α (sTNFR2) were immediately chilled on ice, centrifuged at 4°C, and stored at −70°C until analysis. As soon as all of the samples were collected, they were assayed together in a single assay for each analyte. The adhesion molecules ICAM-1 and VCAM-1 were assayed by ELISA in serum samples. The sTNFR2 level was assayed by enzyme-amplified sensitivity immunoassay (EASIA). All assay kits were obtained from Biosource International.

Insulin sensitivity was assessed by the hyperinsulinemic euglycemic clamp technique [19]. The negative relationships among insulin sensitivity and limb fat and sTNFR2 level in these subjects were reported elsewhere [18]. Body fat distribution was assessed with dual-energy x-ray absorptiometry, as described elsewhere [18]. The percentage of body fat present in the limbs was calculated as the proportion of limb fat to the total of limb and trunk fat, expressed as a percentage.

The Shapiro-Wilk’s test was performed to assess the normality of distribution of each variable. Log transformation was performed for all variables that were not normally distributed. Univariate analyses of variables between control subjects and HIV-infected subjects were performed with Student’s t test. Pearson correlation coefficients were determined for multiple variables. The data were also analyzed with a multivariate regression model in SAS software, version 8.2 (SAS Institute). Statistical significance was determined with 2-tailed tests, and P values <.05 were considered to be statistically significant. Unless otherwise noted, the data are expressed as means ± SEM.

RESULTS

The characteristics of subjects are shown in table 1. Control subjects and HIV-infected subjects were similar in age and sex. HIV-infected subjects had a higher body mass index (calculated as weight in kilograms divided by the square of height in meters), although the mean difference did not reach statistical significance (P = .053). As reported previously, HIV-infected subjects had a significantly smaller proportion of body fat in the limbs (calculated as the level of fat in the limbs divided by the total body fat level and expressed as a percentage) than did control subjects, although body fat normalized to height was not different between control and HIV-infected subjects [18]. Because body fat is apportioned between the limbs and the trunk, the same statistical significance can be attributed to the calculated percentage of trunk fat (calculated as the level of fat in the trunk divided by the total body fat level and expressed as a percentage) as to percentage of limb fat. Insulin sensitivity was significantly lower (P < .001), serum triglyceride levels were higher (P < .01), and HDL cholesterol levels were lower (P < .01) in subjects with HIV disease than in subjects without HIV disease. HIV-infected patients and control subjects did not significantly differ with regard to LDL cholesterol levels, although 3 HIV-infected subjects had elevated triglyceride levels that precluded the measurement of the LDL cholesterol level.

In the HIV disease group, there were 29 subjects (56%) taking protease inhibitors (PIs) at the time of study. Three subjects who were not taking PIs at the time of the study had previously taken PIs. Twenty-four subjects (46%) were taking nonnucleo-
Table 1. Characteristics of HIV-seropositive subjects and HIV-seronegative subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-seronegative subjects (n = 31)</th>
<th>HIV-seropositive subjects (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>37 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>Sex, no. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.2 ± 0.6</td>
<td>27.2 ± 0.7</td>
</tr>
<tr>
<td>CD4+ cell count, cells/mm³</td>
<td>ND</td>
<td>514 ± 42a</td>
</tr>
<tr>
<td>HIV RNA level, median copies/mL range</td>
<td>NA</td>
<td>335 (40–76,000)</td>
</tr>
<tr>
<td>Percentage of limb fat</td>
<td>48.8 ± 1.4</td>
<td>41.5 ± 1.3b,c</td>
</tr>
<tr>
<td>Insulin sensitivity, mg glucose/kg LBM per min</td>
<td>9.98 ± 0.64</td>
<td>6.78 ± 0.48b</td>
</tr>
<tr>
<td>Serum triglyceride level, mg/dL</td>
<td>98 ± 9.36</td>
<td>248 ± 40c,d</td>
</tr>
<tr>
<td>LDL cholesterol level, mg/dL</td>
<td>110 ± 4.1</td>
<td>100 ± 4.1c</td>
</tr>
<tr>
<td>HDL cholesterol level, mg/dL</td>
<td>51 ± 4.1</td>
<td>39 ± 1.5c,d</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SEM, unless otherwise indicated. Body mass index was calculated as weight in kilograms divided by the square of height in meters. HDL, high-density lipoprotein LBM, lean body mass (as determined by dual energy x-ray absorptiometry); LDL, low-density lipoprotein; NA, not applicable; ND, not determined.

* Data are for 50 patients.
* P < .001, by Student’s t test.
* Data are for 47 patients.
* P < .01, by Student’s t test.
* P < .001, by Student’s t test.

Table 2. Plasma concentrations of type 2 soluble receptor for TNF-α (sTNFR2), soluble intercellular adhesion molecule–1 (ICAM-1), and vascular adhesion molecule–1 (VCAM-1).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>HIV-seronegative subjects (n = 31)</th>
<th>HIV-seropositive subjects (n = 52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTNFR2, ng/mL</td>
<td>2.88 ± 0.13</td>
<td>5.11 ± 0.36a</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>282 ± 12</td>
<td>424 ± 23a</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td>821 ± 73</td>
<td>1113 ± 71b</td>
<td>.008</td>
</tr>
</tbody>
</table>

* P < .001, by Student’s t test.
* P < .01, by Student’s t test.

side reverse-transcriptase inhibitors (NNRTIs), and all but 5 subjects (10%) were taking nucleoside reverse-transcriptase inhibitors (NRTIs). Four subjects were not taking any antiretroviral medications at the time of the study, although 3 of these subjects had previously taken PIs, so only 1 subject was naive with respect to antiretroviral therapy.

Serum concentrations of sTNFR2 were significantly higher (P < .001) in HIV-infected patients than in control subjects (table 2). Likewise serum concentrations of ICAM-1 and VCAM-1 were also elevated in individuals with HIV disease, compared with HIV-seronegative control subjects.

Because several variables were not normally distributed, the data were transformed to natural logs (table 3). For each of the simple linear regression models, the natural log of ICAM-1 was the dependent variable, and 15 variables (table 3) were identified as independent variables. The Pearson correlations are given in table 3 for sample sizes ranging from 50 to 83 patients (because of missing data points for some patients). For PI and NRTI, the point-biserial correlation was used because the variables are dichotomous [20]. In univariate analysis, 8 variables were identified as having significant relationships with ICAM-1 level: VCAM-1 level, insulin sensitivity, serum triglyceride level, LDL cholesterol level, HDL cholesterol level, sTNFR2 level, CD4+ cell count, and age. In multivariate analysis, stepwise regression algorithms (determined using SAS software) identified the log of sTNFR2 and the log of VCAM-1 as the only 2 independent predictors of ICAM-1 level. The final model, with only the log of sTNFR2 (P < .001) and the log of VCAM-1 (P = .021) as predictors of the log of ICAM 1 (r² = 0.45), demonstrated normally, identically, and independently distributed residuals.

The relationship of serum sTNFR2 level with ICAM-1 concentration (r = 0.64; P < .001) is shown in figure 1, and the relationship of serum concentrations of sTNFR2 and VCAM-1 (r = 0.48; P < .001) is shown in figure 2. Serum ICAM-1 and VCAM-1 concentrations are also related (r = 0.47; P < .001), as is shown in figure 3.

DISCUSSION

This study demonstrates that circulating levels of the adhesion molecules ICAM-1 and VCAM-1 were elevated in subjects with HIV disease (table 2), as has been reported by others studies.
Table 3. Pearson correlations between the log of soluble intercellular adhesion molecule–1 concentration in serum specimens and other predictors.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>r</th>
<th>P</th>
<th>No. of patients with data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current use of PIs</td>
<td>−0.12</td>
<td>.399</td>
<td>52</td>
</tr>
<tr>
<td>NRTI use</td>
<td>−0.07</td>
<td>.640</td>
<td>52</td>
</tr>
<tr>
<td>Natural log of VCAM-1</td>
<td>0.47</td>
<td>&lt;.0001</td>
<td>83</td>
</tr>
<tr>
<td>Natural log of weight</td>
<td>0.06</td>
<td>.594</td>
<td>83</td>
</tr>
<tr>
<td>Natural log of BMI</td>
<td>0.07</td>
<td>.510</td>
<td>83</td>
</tr>
<tr>
<td>Natural log of viral burden</td>
<td>0.14</td>
<td>.341</td>
<td>50</td>
</tr>
<tr>
<td>Natural log of insulin sensitivity</td>
<td>−0.33</td>
<td>.004</td>
<td>74</td>
</tr>
<tr>
<td>Natural log of serum TG concentration</td>
<td>0.35</td>
<td>.003</td>
<td>68</td>
</tr>
<tr>
<td>Natural log of LDL cholesterol level</td>
<td>0.25</td>
<td>.050</td>
<td>62</td>
</tr>
<tr>
<td>Natural log of HDL cholesterol level</td>
<td>−0.31</td>
<td>.010</td>
<td>68</td>
</tr>
<tr>
<td>Natural log of sTNFR2 concentration</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td>83</td>
</tr>
<tr>
<td>Percentage of limb fat</td>
<td>0.17</td>
<td>.166</td>
<td>72</td>
</tr>
<tr>
<td>CD4+ cell count</td>
<td>−0.29</td>
<td>.041</td>
<td>50</td>
</tr>
<tr>
<td>Height</td>
<td>0.03</td>
<td>.766</td>
<td>83</td>
</tr>
<tr>
<td>Age</td>
<td>0.26</td>
<td>.020</td>
<td>83</td>
</tr>
</tbody>
</table>

NOTE. Protease inhibitor (PI) use and nucleoside reverse-transcriptase inhibitor (NRTI) use are categorical variables (yes/no), and in this case, r is the point-biserial correlation. Missing data reduced the sample size for some variables. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); HDL, high-density lipoprotein; LDL, low-density lipoprotein; sTNFR2, type 2 soluble receptor for TNF-α; TG, triglyceride; VCAM-1, vascular adhesion molecule–1.

Although the increase in levels of adhesion molecules could be related to multiple variables—including insulin resistance, changes in body fat distribution (i.e., percentage of limb fat), and serum lipid levels (i.e., triglyceride levels) (table 3)—multivariate analysis, with partial correlation, which we obtained by controlling for the impact of these variables on inflammation (i.e., sTNFR2), suggested that only inflammation had an independent effect on the levels of adhesion molecules. This important finding suggests that circulating levels of adhesion molecules—and, by implication, an increased risk of cardiovascular disease—may be associated with inflammation, as indexed by the sTNFR2 level in subjects with HIV disease.

The increase in sTNFR2 level in patients with HIV disease is in accord with the findings of previous studies [18, 21], but the impact of inflammation on other variables, such as insulin resistance and changes in body fat distribution, may help to provide a paradigm to unify a number of disparate observations. For example, Hadigan et al. [22] showed that the increased risk of coronary heart disease in subjects with HIV disease is associated with altered body fat distribution. We have shown that alterations in body fat distribution in HIV-infected subjects are also related to inflammation [18]. Other studies have suggested that use of HAART led to decreased concentrations of circulating adhesion molecules, compared with data for HIV-infected individuals who were HAART naive [17], suggesting that drug treatment could improve endothelial cell function. However, other studies have suggested that antiretroviral medications have a negative impact on endothelium [23, 24]. Not only has treatment with PIs been associated with endothelial cell damage, but so has treatment with NNRTIs [25, 26]. Although assessment of the impact of individual medications in isolation is important to understanding potential drug pathology, it may also be instructive to compare the effects of drug regimens on inflammation. The drug regimens that reduce inflammation may also be those associated with reductions in the circulating levels of adhesion molecules.

Likewise, dyslipidemia is associated with increased circulation of endothelial adhesion molecules [25]. PI use is also associated with an elevation in serum lipid levels [27], and in a study of ~17,000 subjects, Friis-Moller et al. [28] reported that both PI use and NNRTI use resulted in a lipid profile that indicated increased cardiovascular risk. That these effects on lipid levels in HIV disease may be mediated through inflammation was suggested in a study by Grunfeld et al. [29], who demonstrated that dyslipidemia in HIV-infected subjects is associated with the upregulation of the TNF system.

The importance of inflammation in elevated levels of adhesion molecules is also suggested by studies of obese subjects. Obesity was associated with elevated levels of ICAM-1, compared with levels in nonobese subjects [30], and reductions in ICAM-1 levels occurred with weight loss due to calorie restriction [30]. Like the HIV-infected subjects in the present study, the levels of ICAM-1 in obese subjects were associated with insulin resistance and activation of the TNF-α system [31]. However, not all of the potential factors that may have an impact on inflammation (e.g., smoking [32]) were assessed in the present study.

Soluble forms of adhesion molecules, such as ICAM-1 and VCAM-1, are present in the circulation and have been used as indicators of endothelial cell damage, but so has treatment with NNRTIs [25, 26]. Not only has treatment with PIs been associated with endothelial cell damage, but so has treatment with NNRTIs [25, 26]. Although assessment of the impact of individual medications in isolation is important to understanding potential drug pathology, it may also be instructive to compare the effects of drug regimens on inflammation. The drug regimens that reduce inflammation may also be those associated with reductions in the circulating levels of adhesion molecules.

Figure 1. The relationship of soluble receptor type 2 for TNF-α (sTNFR2) with soluble intercellular adhesion molecule–1 (ICAM-1) in subjects with HIV disease. The figure shows the linear regression of the concentration of sTNFR2 with ICAM-1 in subjects with HIV disease (■) and in HIV-seronegative control subjects (●; r = 0.64; P < .001).
markers for cardiovascular disease in subjects without HIV disease [9–12]. Although the present study is only a small study, it does suggest that a larger study would be of value in substantiating the role of inflammation in cardiovascular disease associated with HIV disease. A longitudinal study of cardiovascular disease that observes previously HAART-naive patients during treatment with antiretroviral medications would confirm the hypothesis that inflammation is responsible for the increased levels of adhesion molecules, which are indicative of increased risk of cardiovascular disease, in subjects with HIV disease. Other disease parameters, such as duration of disease, nadir CD4+ cell count, and changes in CD4+ cell counts, could also be investigated for effects on levels of adhesion molecules and on cardiovascular disease and to determine whether these effects are independent of effects on inflammation. In addition to parameters related to disease and treatment, HIV-infected individuals are also susceptible to the multiple parameters that impact the risk of cardiovascular disease seen in individuals without HIV disease—factors that also have an impact on inflammation, such as smoking [33]; diet quality [34], including consumption of trans-fatty acids [35] and antioxidants [36]; level of physical activity [37]; and genetic polymorphisms [38, 39]. What the present study suggests is that future studies of the risk factors for cardiovascular disease need to consider to what degree these factors pose a risk that is independent of their effect on inflammation.

In conclusion, although HAART is associated with improved longevity in patients with HIV disease as a result of increased CD4+ cell counts and decreased viral burdens, this improvement in disease progression is accompanied by increased risk of cardiovascular disease. Markers such as ICAM-1 and VCAM-1 concentration can help identify patients with an increased risk of cardiovascular disease. But even more importantly, markers of inflammation, such as sTNFR2, can be used to identify patients who are most at risk of developing cardiovascular disease. Therefore, in the era of HAART, all HIV-infected patients should be assessed for the traditional risk factors for cardiovascular disease, and if such factors are present, plans to modify these risk factors (including reductions in inflammation) should be considered.

Acknowledgments
We acknowledge the help of Dr. Mark Kaplan from the Department of Infectious Diseases, North Shore University Hospital, and Dr. Roy Steinberg at Stony Brook in recruiting subjects from their clinical practice. We also thank the nursing staff of the General Clinical Research Center for meticulously carrying out this protocol. The careful technical assistance from GuiQin Yu is gratefully acknowledged, as is the help of Drs. Joshua Feiner and Robert Ferris in the preparation of the manuscript.

Financial support. National Institutes of Health (DK49316 to M.C.G. and MO1 RR10710, which supports the General Clinical Research Center at Stony Brook) and the Empire Clinical Research Investigators Program Award (to M.M.M.).

Potential conflicts of interests. All authors: no conflicts.

References


