Atherosclerosis in Patients Infected With HIV Is Influenced by a Mutant Monocyte Chemoattractant Protein-1 Allele

Carlos Alonso-Villaverde, MD; Blai Coll, MD; Sandra Parra, MD; Manuel Montero, MD; Nahum Calvo, MD; Mónica Tous, PhD; Jorge Joven, MD; Lluis Masana, MD

Background—Patients infected with HIV present with premature atherosclerosis, and the 2 diseases share common pathogenic pathways. We investigated mutations in the monocyte chemoattractant protein-1 (MCP-1) and CCR-2 genes, which are known to control aspects of these pathways, to ascertain whether they are involved in atherogenesis in these patients.

Methods and Results—We performed carotid and femoral artery ultrasonography to detect subclinical atherosclerosis in patients infected with HIV (n=183). MCP-1–2518G and CCR-2 64I polymorphisms were determined in the HIV group and in a population-based control group (n=348). We also determined MCP-1 circulating levels in the HIV group. The presence of MCP-1–2518G in the group of patients with subclinical atherosclerosis was significantly higher than in patients without atherosclerotic lesions (47.5% versus 18.2%, respectively; P<0.001). Furthermore, the patients with atherosclerotic lesions had higher MCP-1 plasma concentrations than did patients without lesions (74.15 [4.03] versus 57.81 [3.67] pg/mL, respectively; P=0.03). When adjusted for known cardiovascular risk factors, the MCP-1–2518G allele was associated with subclinical atherosclerosis (OR 5.72, 95% CI 1.74 to 18.80, P=0.004). Compared with measurements conducted ≥2.5 years earlier in a subset of 40 patients, intima-media thickness (IMT) in the carotid artery progressed at a mean rate of 0.06 mm/y more rapidly in patients bearing the MCP-1–mutated allele (P=0.08).

Conclusions—HIV-infected patients with the MCP-1–2518G allele have a 5-fold increased risk for atherosclerosis, as assessed by ultrasonography. (Circulation. 2004;110:2204-2209.)

Key Words: atherosclerosis ■ HIV ■ inflammation ■ genotype ■ prevention
Some HIV proteins can induce the overexpression of MCP-1, and the levels of this protein may be altered during the course of HIV progression.11–25 This disease feature is exacerbated in carriers of the MCP-1 mutant allele. As such, the evidence to date suggests that HIV infection and atherosclerosis share pathways in their pathogenesis. It is a reasonable hypothesis that mutations in genes that control aspects of these pathways could affect the course of both diseases. Hence, we assessed whether known associations between MCP-1 and CCR-2 mutant alleles and atherosclerosis in the general population also are found in an HIV-infected population.

Methods

Study Design
We performed a case-control study based on the presence or absence of atherosclerosis in 183 subjects infected with HIV. We also evaluated clinical, laboratory, and genotyping results in the cases and controls to assess the risk factors for atherosclerosis in this particular clinical setting. For genotype comparisons we used a general population–based control group of unrelated subjects (n=348), the details of which have been described elsewhere.26

Study Participants and Eligibility
From among the patients infected with HIV who attended our clinic (n=305), 183 accepted the invitation to participate in the study and provided fully informed consent. Among the exclusion criteria were being <18 years old, having AIDS-related opportunistic diseases at the commencement of the study, and declining the invitation to participate. The Ethics Committee of the Hospital Universitari de Sant Joan de Reus approved the study.

Outcome Measurements

Clinical and Laboratory Measurements
A detailed clinical record was taken of each subject and a thorough physical examination was performed at interview. The traditional cardiovascular risk factors assessed were smoking status, presence or absence of hypertension, and body mass index (defined as the weight in kilograms divided by the square of the height in meters). A sample of fasting venous blood was taken for the measurement of glucose, total cholesterol, HDL cholesterol, and triglycerides. The analyses were conducted using standard laboratory methods. The LDL cholesterol level was calculated using the Friedewald formula.

Ultrasonography Measurements
Ultrasonography to measure intima-media thickness (IMT) was performed as previously described27 with a LOGIQ 700 MR system (General Electric). When a plaque was identified at a predefined point, the IMT was determined in adjacent segments. The presence of atherosclerosis was defined as IMT >0.8 mm, the presence of a plaque, or both27 in either carotid or femoral territories. We used this selection criterion to define the subject as a case or as a control. The concordance between the 2 sonographers responsible for the atherosclerosis evaluations indicated a high correlation coefficient of \( \kappa >0.8 \) for the independently conducted measurements.

Inflammatory Marker Measurements
Venous blood samples were collected into EDTA-containing tubes. The concentration of C-reactive protein (CRP) was measured by a particle-enhanced turbidimetric immunoassay (Quantex hs-CRP kit, Biokit), which had a sensitivity of 0.10 mg/L. The plasma concentration of MCP-1 was measured according to the manufacturer’s recommendations with an enzyme-linked immunosorbent assay (Human MCP-1 ELISA Development Kit, PeproTech), which had a measurement range of 8 to 3000 pg/mL.

Genotyping
DNA was extracted by a standard phenol-chloroform procedure. The mutations MCP-1–2518G and CCR-2 64I were identified according to previously published methods.29

Risk Factor Analysis
Multivariate logistic regression analyses were performed to adjust for known cardiovascular risk factors. The data on carotid, femoral, or carotid and femoral atherosclerosis were the dependent variables, and the independent variables included age, sex, smoking habit, blood pressure, lipid profile, plasma glucose, mean duration of each antiretroviral treatment (ie, protease inhibitor, non-nucleoside inhibitors, and nucleoside analogues) and the DNA polymorphisms.

Atherosclerosis Progression
In a pilot study conducted \( \approx 3 \) years previously, 40 patients were examined and clinical and ultrasonography data were documented. The stored images were retrieved and compared with the present measurements to assess atherosclerosis status. Comparisons included the mean IMT change (in millimeters) of predefined carotid arterial segments and the change (in square millimeters) in the area of a previously selected carotid plaque. These data were used to assess changes in the dimensions of the arterial lesions during the specific period.

Statistical Analyses
Data are presented as means with the standard error of the mean in parentheses. Standard methods (Kolmogorov-Smirnov and Shapiro-Wilk tests) were used to check for normality of the distributions. Analysis of variance was used to compare differences in quantitative variables, and the \( \chi^2 \) test was used for categorical variables. Allele frequencies were calculated by the gene-counting method. The Hardy-Weinberg equilibrium and the differences in biallelic polymorphisms (genotype distributions and allele frequencies) between groups were tested using the \( \chi^2 \) test. Analysis of variance was used to compare changes in mean IMT and the area of the plaque over time. The significance of association between the MCP-1 allele and the increase in the variables was assessed using a multiple linear regression model in which adjustment was made for other conventional cardiovascular risk factors. All probability values \( P<0.05 \) were considered to be statistically significant. All analyses were performed with SPSS statistical software (version 11.0).

Results
We studied 183 HIV-infected subjects (124 men, 59 women, 20 to 66 years old). The subgroup of 40 HIV–infected subjects (34 men, 6 women, 32 to 58 years old) used for assessing atherosclerosis progress did not present any clinical differences when compared with the overall HIV study group. When known cardiovascular risk factors were compared (age, lipid profile, or patients with high blood pressure or abnormal fasting glucose), we did not find any statistically significant differences.

Subclinical Atherosclerosis and Control Groups
The measured variables, including conventional cardiovascular risk factors and segregated with regard to presence of atherosclerosis, are presented in Table 1. Subjects were of the same ethnic (white) background. The participants with atherosclerosis were significantly older than the control group \( (P<0.001) \). We evaluated conventional cardiovascular risk factors between groups. Most subjects were heavy smokers. Although we did not find differences in the mean body mass index (BMI) when cases and controls were compared \( (23.10 [0.27] \text{ versus } 23.00 [0.52], \text{respectively}; P=0.857) \), we found higher rates of hypertension and abnormal fasting glucose.
concentrations in the subjects who had atherosclerosis. Only 7 of these subjects were receiving statin therapy (for <1 year), and fibrates had been used in 10 subjects during the previous 6 months. None of the participants included in the study presented with either cardiac or cerebral ischemic events. We did not find any statistically significant differences with regard to HIV-related variables such as baseline CD4 cell count, AIDS-related opportunistic disease, or the time lapse after HIV diagnoses. Seven cases (5.0%) and 5 controls (11.4%) were naïve with regard to antiretroviral therapy (P=0.163). Segregation by sex, age, or both did not affect the distribution of genotypes; thus, all subjects were analyzed as a single group. The allelic distribution of MCP-1 and CCR-2 genotypes followed the Hardy-Weinberg equilibrium (χ², P=0.30 and P=0.81, respectively) in patients in the case group as well as in controls. No statistically significant differences between the unrelated subject control group and either of the case groups with regard to genotype distributions or in allelic frequencies were found (Table 2). Also, no differences were found in the distributions of both the mutations.

**Analysis of Subclinical Atherosclerotic Lesions**

The majority of subjects infected with HIV (n=139; 76.0%) presented with atherosclerotic lesions in one or another of the territories assessed, a percentage that is equivalent to that observed in other similar studies. Analysis of the distribution of genotypes according to the presence or absence of subclinical atherosclerosis indicated that the frequencies of GG and GA genotypes in the MCP-1 polymorphism were significantly higher in subjects with atherosclerosis than in those without (47.5% versus 18.2%; P=0.001). The results showed that subjects with at least 1 mutated allele were more likely to show evidence of atherosclerosis (89.2%). No differences were observed in the distribution of CCR-2 polymorphism between subject populations (Table 2).

It is worth noting that our study population was relatively young, with >75% of subjects <42 years old. An analysis of

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**TABLE 1. Characteristics of HIV-Infected Patients Segregated According to Presence or Absence of Atherosclerosis**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Atherosclerosis (n=139)</th>
<th>No Atherosclerosis (n=44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>40.71 (0.59)</td>
<td>34.15 (0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, %</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Male</td>
<td>71.9</td>
<td>54.5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28.1</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>Conventional cardiovascular risk factors,* %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>82.6</td>
<td>88.6</td>
<td>0.32</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15.6</td>
<td>2.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Abnormal fasting glucose</td>
<td>14.4</td>
<td>...</td>
<td>0.01</td>
</tr>
<tr>
<td>Dyslipemia</td>
<td>27.0</td>
<td>16.7</td>
<td>0.16</td>
</tr>
<tr>
<td>Lipid profile, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.06 (0.12)</td>
<td>4.77 (0.16)</td>
<td>0.22</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.85 (0.09)</td>
<td>2.63 (0.12)</td>
<td>0.22</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.16 (0.04)</td>
<td>1.28 (0.06)</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2.44 (0.18)</td>
<td>1.82 (0.28)</td>
<td>0.09</td>
</tr>
<tr>
<td>Risk factor for HIV infection</td>
<td></td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>Intravenous drug use, %</td>
<td>59.0</td>
<td>52.3</td>
<td></td>
</tr>
<tr>
<td>Male homosexual contact, %</td>
<td>9.7</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Heterosexual contact, %</td>
<td>31.3</td>
<td>34.1</td>
<td></td>
</tr>
<tr>
<td>Months since HIV diagnosis</td>
<td>89.25 (4.34)</td>
<td>78.48 (7.83)</td>
<td>0.22</td>
</tr>
<tr>
<td>Basal CD4, %</td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;500</td>
<td>35.3</td>
<td>34.1</td>
<td></td>
</tr>
<tr>
<td>200–500</td>
<td>44.6</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>20.1</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Previous antiretroviral therapy, mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleoside analogs</td>
<td>100.87 (5.41)</td>
<td>85.25 (8.74)</td>
<td>0.15</td>
</tr>
<tr>
<td>Protease inhibitor</td>
<td>29.70 (2.39)</td>
<td>26.06 (3.78)</td>
<td>0.44</td>
</tr>
<tr>
<td>Non-nucleoside reverse transcriptase inhibitors</td>
<td>8.35 (0.89)</td>
<td>8.70 (1.69)</td>
<td>0.85</td>
</tr>
<tr>
<td>AIDS-related disease, %</td>
<td>32.4</td>
<td>31.8</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Hypertension defined as >140, >90 mm Hg, or both. Abnormal fasting glucose defined as fasting plasma glucose >6.1 mmol/L. Dyslipemia defined as LDL cholesterol >3.36 mmol/L.
the data segregated with regard to age quartiles indicated that subjects with at least 1 mutated allele for MCP-1 had higher rates of atherosclerotic lesions in each of the quartiles (Figure 1). This finding was especially relevant in subjects <34 years old.

We then analyzed the association between atherosclerosis and inflammatory markers such as CRP and MCP-1. Although no significant differences were found among MCP-1 plasma concentrations according to the MCP-1 polymorphism, a higher concentration was observed when subjects with HIV and atherosclerosis were compared with those without any arterial lesions (74.15 [4.03] versus 57.81 [3.67] pg/mL, respectively; \( P < 0.03 \)). We did not find significant differences in CRP concentrations between subjects with atherosclerosis and those without (3.38 [0.31] versus 3.46 [0.54] mg/mL, respectively; \( P = 0.905 \)).

Multivariate logistic regression analysis with known cardiovascular risk factors as independent variables revealed that only age and the MCP-1–2518G polymorphism were significantly associated with the presence of subclinical atherosclerosis (Figure 2). Treatments with protease inhibitor or non-nucleoside–based regimens were not associated with the presence of subclinical atherosclerosis (\( P = 0.64 \) and \( P = 0.56 \), respectively). Age was significantly associated with atherosclerotic lesions (OR 1.32, 95% CI 1.17 to 1.50, \( P < 0.001 \)) as was the MCP-1–2518G allele (OR 5.72, 95% CI 1.74 to 18.80, \( P = 0.004 \)).

The effect of the MCP-1–mutated allele was evaluated with respect to the clinical course of the atherosclerotic lesions with the stored images available for 40 subjects. The time lapse between the 2 ultrasonographic measurements was 2.61 (0.07) years. When subjects were segregated into those with the MCP-1–2518G (\( n = 13 \)) allele and those with the AA genotype (\( n = 27 \)), the subjects with the mutated allele appeared to have a poorer clinical outcome (Figure 3). The data indicated an increase in carotid IMT of 0.06 mm/y in the subject group with the mutated allele (MCP-1–2518G). In the group of subjects with the AA genotype, this increase was 0.03 mm/y; however, the difference did not reach statistical significance (\( P = 0.08 \)). When the areas of predefined carotid lesions were analyzed it was apparent that subjects with at least 1 mutated allele experienced a significantly higher increase than did subjects with the wild-type allele (12.9 [4.3] versus 32.3 [6.4] mm², respectively; \( P = 0.04 \)).

**Discussion**

Some genetic variants of the chemokines are reputed to influence individuals’ susceptibility to HIV infection, the progression of the disease, and even the presence of so-called HIV-associated manifestations.\(^{28}\) Considerable research has focused on the role of chemokine polymorphic genes implicated in the inflammatory response and, as a consequence, in atherosclerosis.\(^{29}\)

An important finding of our study is that a mutation in the promoter region of the MCP-1 gene has an atherosclerosis-promoting effect. Infiltration of tissues by monocyte-derived

**Table 2. Distribution of Genotypes and Inflammatory Markers Among HIV-Infected Patients With or Without Subclinical Atherosclerosis**

<table>
<thead>
<tr>
<th>Atherosclerosis (n=139)</th>
<th>No atherosclerosis (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCP-1–A2518G genotype distribution, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>GG + GA</td>
<td>66 (47.5)</td>
</tr>
<tr>
<td>AA</td>
<td>73 (52.5)</td>
</tr>
<tr>
<td><strong>CCR-2–A190G genotype distribution, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>GG + GA</td>
<td>25 (18.0)</td>
</tr>
<tr>
<td>AA</td>
<td>114 (82.0)</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>3.38 (0.31)</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>74.15 (4.03)</td>
</tr>
</tbody>
</table>

*\( P < 0.001 \).
†\( P = 0.71 \).
‡\( P = 0.03 \).

**Figure 1.** Percentage of subjects infected with HIV with subclinical atherosclerosis according to MCP-1–2518G (■) and –2518A (■) polymorphism and distributed by quartiles of age. Logistic regression model was applied to assess association of carotid and femoral atherosclerosis with presence of MCP-1–mutated allele adjusted for age (\( P < 0.001 \)).

**Figure 2.** ORs for subclinical atherosclerosis. Horizontal lines represent 95% CI. * indicates that OR is higher than represented. (See Methods for further information.) *\( P < 0.001 \); †\( P = 0.004 \).
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previous studies, atherosclerosis was less extensive in polymorphism.

... pollyestrogen with regard to MCP-1 polymorphism. Differences were calculated between the 2 ultrasonographs conducted on 40 subjects and segregated with regard to MCP-1 polymorphism. Figure 3. Change in IMT and area of predefined atheromatous plaque of internal carotid artery. Differences were calculated between the 2 ultrasonographs conducted on 40 subjects and segregated with regard to MCP-1 polymorphism.

macrophages is a prominent feature of atherosclerosis. MCP-1 and possibly other chemoattractant proteins are thought to be the molecular signals that direct such infiltration. We measured plasma MCP-1 concentrations and, despite these individuals’ being subject to multiple infections and inflammatory insults and receiving antiretroviral therapies that can induce further changes in MCP-1 levels, we found an association between higher values of MCP-1 and atherosclerosis. This finding provides support for our hypothesis that MCP-1 may play a crucial role in atherogenesis. In previous studies, atherosclerosis was less extensive in patients who had well-established cardiovascular disease and who carried the mutation in the CCR-2 gene. We are unable to confirm such results based on our multivariate analyses. We wish to highlight that with regard to conventional cardiovascular risk factors, subjects with atherosclerosis experienced higher rates of hypertension and abnormal fasting glucose. In multivariate analyses, these variables lost their statistical significance in relation to atherosclerosis.

Ultrasonography, being a noninvasive tool, is widely accepted in the evaluation of IMT, and IMT has been validated as a surrogate marker for atherosclerotic vascular disease. For example, a yearly increase of carotid artery IMT of 0.03 mm is associated with an increase in coronary events in patients with established atherosclerosis. Conversely, the reduction of 0.03 mm/y achieved with high-dose statins appears to have a significant impact on the prevention of coronary artery disease. Although the present statistical analyses are not a case-control study of “atherosclerosis” versus “no-atherosclerosis” comparisons, the yearly increase of carotid IMT in our subjects was clearly >0.03 mm and was more evident in subjects with the MCP-1–2518G allele. Although drug interactions, toxicity, intolerance, and decreased adherence to treatment are common in these subjects, we believe our data suggest that the prescription of statins, fibrates, or both in subjects with HIV could induce favorable outcomes with regard to the development of atherosclerosis in these subjects.

In summary, our results indicate that the MCP-1–CCR-2 gene axis is related to carotid and femoral atherosclerosis in patients infected with HIV. These findings need to be reflected in proposals for new therapies. For example, an increase in the prescription of statins, platelet antiaggregants, or both together with the use of antiretroviral regimes would be appropriate. Conversely, the inducers of metabolic disturbances would need to be reduced to minimize the risk of vascular events in these patients. Knowledge of the activation mechanisms of chemokines in HIV and other inflammatory disorders would provide insight into better management and control of HIV-associated diseases, including atherosclerosis.

Acknowledgments
This study was supported by grants from Fundación Española de Arteriosclerosis and the Instituto de Salud Carlos III, RCMN (C03/08), Madrid, Spain. The authors are indebted to Alberto Amejide for statistical support and Asunción González for her invaluable nursing assistance.

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_Circulation_. 2004;110:2204-2209; originally published online October 4, 2004;
doi: 10.1161/01.CIR.0000143835.95029.7D
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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