

Circulating Endothelial Progenitor Cell Levels Are Not Reduced in HIV-Infected Men

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Reduced levels of endothelial progenitor cells (EPCs) have been associated with increased cardiovascular (CV) risk, but limited data are available on EPC levels in the human immunodeficiency virus (HIV)-infected population. EPCs (CD45^{dim}/CD34⁺/kinase domain receptor⁺) from 36 HIV-uninfected and 30 antiretroviral therapy-naïve HIV-infected men without known CV risk factors were enumerated using flow cytometry. The mean EPC levels (\pm standard error of the mean) were 1.4 ± 0.5 cells/ μ L in the HIV-infected group and 3.7 ± 2.2 cells/ μ L in the control group ($P = .92$). EPC levels were not associated with disease parameters, such as CD4 cell count or viral load. Reductions in EPC levels do not seem to explain the increased risk of CV disease among HIV-infected men.

Epidemiologic studies have shown that rates of cardiovascular (CV) events among human immunodeficiency virus (HIV)-infected individuals may be increased by 3-fold, compared with the general population [1], thus making CV disease a significant threat to longevity in HIV-infected individuals. Because endothelial dysfunction is an early marker of atherosclerosis and carries predictive value for CV events, it has been suggested that endothelial dysfunction may provide a link between HIV infection and atherosclerosis. Furthermore, it has already been

demonstrated that endothelial function is impaired at various stages in HIV infection [2].

Endothelial progenitor cells (EPCs) are a circulating progenitor population thought to be involved in vascular homeostasis. For example, they play a role in endothelial repair by homing to sites of injury and directing repair either through direct incorporation into the endothelium or by secreting proangiogenic paracrine factors [3]. Hill et al [4] first demonstrated a correlation between the number of circulating EPCs and patients' combined Framingham risk scores. Specifically, they demonstrated that levels of circulating endothelial cells were a superior predictor of vascular reactivity, compared with the presence or absence of conventional risk factors, and suggested that levels of EPCs may be a surrogate biologic marker for vascular function and cumulative CV risk. These findings have been corroborated by others, and it is well established that patients with reduced levels of EPCs have more frequent CV events [5].

Our objective was to determine whether there is a difference in EPC levels between HIV-infected and uninfected men. If a difference between these 2 populations were to be demonstrated, this may provide new insight into the mechanisms by which HIV infection accelerates atherosclerosis.

METHODS

Participants

Patients were highly active antiretroviral therapy (HAART)-naïve HIV-infected men attending the Ottawa Hospital Immunodeficiency Clinic in Ottawa, Ontario, or the Maple Leaf Medical Clinic in Toronto, Ontario. Control subjects were HIV-uninfected men recruited from the same sites. Patients and control subjects were enrolled on a consecutive basis. Inclusion criteria included age of 20–40 years and either having never smoked or abstinence from smoking for ≥ 3 months before enrollment. Exclusion criteria were selected to exclude medical conditions or medications known to influence EPC levels. These included a history of coronary artery disease, myocardial infarction, symptoms associated with atherosclerosis or active ischemia, stroke or symptoms suggestive of transient ischemic attack, diabetes, hypertension, dyslipidemia, conditions in which neovascularization may be present (cancer or retinopathy), hepatitis B or C, or any other chronic infection or acute illness. Use of medications known to influence EPC levels, including statins, angiotensin converting enzyme (ACE) inhibitors, glitazones, or granulocyte-colony stimulating factor,

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was also an exclusion criteria. We included only men in this small study, because estradiol dose dependently increases levels of EPCs, and circulating progenitor populations vary throughout the menstrual cycle.

All participants underwent a detailed assessment of cardiac risk and physical examination, including blood pressure, height, and weight measurements. All individuals had blood samples collected for complete blood cell count, fasting lipid profile, blood glucose level, erythrocyte sedimentation rate (ESR), and C-reactive protein measurement. In HIV-infected individuals, CD4 cell count and plasma viral load were measured if values obtained within the previous 3 months were not available. Whole blood was collected in ethylenediaminetetraacetic acid for EPC analysis. Approval was obtained from the respective institutional research ethics boards in advance, and all participants provided written informed consent.

EPC Enumeration

Blood samples were analyzed within 24 hours after collection with use of 5-color flow cytometry. In brief, EPCs were pre-defined as CD45^{dim}/CD34⁺/kinase insert domain receptor (KDR)⁺ based on currently accepted definitions [6, 7]. We also chose to examine CD45^{dim}/CD34⁺/CD117⁺ and CD45^{dim}/CD34⁺/CD184⁺ progenitor populations, because CD117⁺ and CD184⁺ are the cognate receptors for stem cell factor and stromal derived factor-1, respectively, both of which have been shown to have important roles in EPC function. Whole blood underwent red blood cell lysis with IOTEST 3 lysing solution (Beckman Coulter). Antibodies for CD45–Pacific Blue (Beckman Coulter; clone J.33), CD117-PC7 (phycoerythrin cyanin 7) (Beckman Coulter; clone 104D2D1), CD184-APC (allophycocyanin) (BD Pharmingen; clone 12G5), CD133-PE (phycoerythrin) (Miltenyi Biotec; clone AC133), and CD34-ECD (Phycoerythrin-Texas Red) (Beckman Coulter; clone 581) were used in this study. All flow cytometry was performed on a Cyan ADP9 cytometer (Beckman Coulter), and data were analyzed using Kaluza (version 1.1; Beckman Coulter). All flow cytometric data collection and analysis were performed in a central laboratory by an investigator blinded to the serostatus of the study participants.

Statistical Analysis

The sample size was calculated on the basis of the results of Vasa et al [8]. With an α of .05 and a power of 90%, we determined that 23 individuals per group would be required to detect a 50% decrease in mean EPC level in HIV-infected individuals. We sought to enroll a minimum of 30 individuals per group to facilitate the study of potential correlates of EPC levels in HIV-infected persons. Baseline characteristics are expressed as means \pm standard deviations and EPC data are expressed as means \pm standard error of the mean. For normally distributed data, comparisons were performed using a 2-tailed *t* test. For

Table 1. Baseline Characteristics of HIV-Infected Patients and Control Subjects

Characteristic	HIV-Infected Patients (n = 30)	Control Subjects (n = 36)	<i>P</i>
Age, y	33 \pm 6	30 \pm 4	.05
BMI	25.56 \pm 3.96	24.61 \pm 2.52	.15
TG, mmol/L	1.54 \pm 0.71	1.13 \pm 0.65	.01
HDL, mmol/L	0.98 \pm 0.27	1.32 \pm 0.25	.01
LDL, mmol/L	2.43 \pm 0.86	2.66 \pm 1.06	.41
Total cholesterol, mmol/L	4.07 \pm 0.92	4.47 \pm 1.14	.15
Fasting glucose, mmol/L	4.49 \pm 0.97	4.18 \pm 0.81	.29
ESR, mm/h	10.32 \pm 17.85	3.89 \pm 6.92	.02
CRP, mg/L	1.96 \pm 2.53	1.94 \pm 4.06	.91
Blood pressure, mm Hg			
Systolic	125 \pm 13	120 \pm 9	.05
Diastolic	70 \pm 7	73 \pm 8	.09
Family history of premature CAD, No. (%)	6 (20)	4 (11)	.42
Race/ethnicity, No. (%)			
White	18 (60)	25 (69)	.59
Other	12 (40)	11 (31)	.59
HIV parameters			
CD4 cell count, cells/ μ L	478 \pm 303
CD4 cell percentage	25.1 \pm 8.5
CD4-CD8 cell ratio	0.56 \pm 0.33
Viral load, log ₁₀ copies/mL	3.86 \pm 1.01
Time since HIV diagnosis, y	3.0 \pm 2.3

Data are means \pm standard deviations, unless otherwise indicated.

Abbreviations: BMI, body mass index (measured as weight in kilograms divided by square of height in meters); CAD, coronary artery disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; TG, triglyceride.

nonparametric statistical testing, a Mann–Whitney rank sum test was used. For correlational analysis, Pearson correlation coefficients are reported. All statistical testing was performed using Sigma Stat software (version 3.5).

RESULTS

Baseline Clinical and Laboratory Profiles

Samples were obtained from 30 HIV-infected men and 36 healthy control subjects. Twenty-two HIV-infected men and 28 HIV-uninfected control subjects were enrolled from The Ottawa Hospital, and 8 HIV-infected and 8 HIV-uninfected men were enrolled from the Maple Leaf Medical Clinic. HIV-infected men had a significantly higher mean triglyceride level than did control subjects (1.54 \pm 0.71 vs 1.13 \pm 0.65 mmol/L; *P* = .009) and a higher mean ESR than did control subjects (10.32 \pm 17.85 vs 3.89 \pm 6.92 mm/h; *P* = .015). Otherwise, the 2 groups were similar (Table 1). The mean CD4 cell count and CD4 cell percentage in HIV-infected men at the time of study

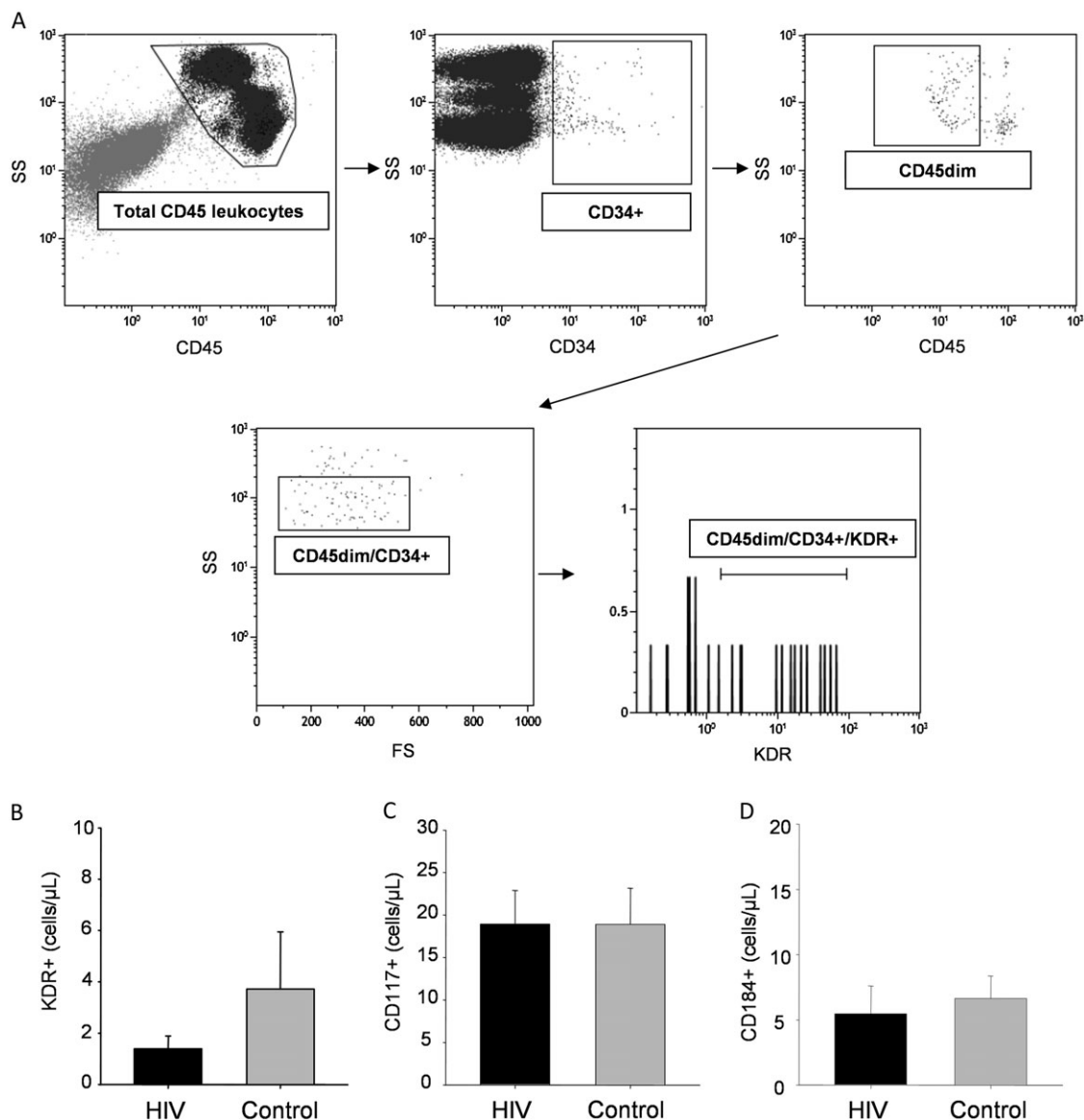


Figure 1. Circulating endothelial progenitor cell (EPC) levels are not reduced in human immunodeficiency virus (HIV) infection. *A*, Representative gating strategy used for rare cell analysis. *B*, CD45^{dim}/CD34⁺/KDR⁺ cells in HIV-infected and healthy men. *C*, CD45^{dim}/CD34⁺/CD117⁺ cells in HIV-infected and healthy men. *D*, CD45^{dim}/CD34⁺/CD184⁺ cells in HIV-infected and healthy men. Data are presented as means \pm standard error of the mean ($n = 30$ for HIV-infected patients and $n = 36$ for control subjects; $P > .05$ for all comparisons).

enrollment were 478 ± 302 cells/ μ L and $25\% \pm 8.5\%$, respectively. The mean CD4-CD8 cell ratio was 0.56 ± 0.33 . The geometric mean viral load at study enrollment was 3.86 ± 1.01 log₁₀ copies/mL, and the mean time from diagnosis of HIV infection to enrollment was 3 ± 2.3 years.

EPC Levels in HIV-Infected Men, Compared With Healthy Control Subjects

We a priori defined EPCs in this study as CD45^{dim}/CD34⁺/KDR⁺ cells and enumerated them using a validated modification of the International Society of Hematology and

Graft Engineering (ISHAGE) protocol (Figure 1A) [7]. With use of this definition, mean EPC levels were 1.4 ± 0.5 cells/ μ L in the HIV-infected group, which was not significantly different from those in the control group (3.7 ± 2.2 cells/ μ L; $P = .92$) (Figure 1B). We also analyzed levels of CD45^{dim}/CD34⁺/CD184⁺ and CD45^{dim}/CD34⁺/CD117⁺ as a secondary end point. Similar to KDR⁺ cells, levels of CD117⁺ progenitors (30.0 ± 7.3 vs 35.1 ± 5.4 cells/ μ L; $P > .05$) (Figure 1C) and CD184⁺ progenitors (5.5 ± 2.2 vs 6.6 ± 1.7 cells/ μ L; $P > .05$) (Figure 1D) did not differ between HIV-infected men and control subjects. In the overall cohort, EPC level did not

correlate with age, C-reactive protein level, ESR, or either CD184⁺ or CD117⁺ progenitor populations. Recently, da Silva et al [9] reported lower numbers of CD34⁺/KDR⁺ cells in HIV-infected HAART-naive patients. We replicated their method of EPC enumeration by excluding granulocytes (as would be lost with Ficoll isolation) and enumerated CD34⁺/KDR⁺ cells as a percentage of the remaining cells. As with our established definition of EPCs, the proportions of CD34⁺/KDR⁺ cells did not differ between HIV-infected patients (0.006 ± 0.001) and healthy control subjects (0.012 ± 0.003 ; $P = .273$).

EPC Levels and Parameters of HIV Infection

Although no differences existed between healthy control subjects and HIV-infected individuals, we performed an exploratory analysis examining disease-related parameters and EPC levels. Specifically, we wished to determine whether any relationships existed between absolute CD4 cell count, log₁₀ viral load, and time since diagnosis of HIV infection with EPC levels. In our cohorts, no correlation could be established between any of the aforementioned parameters and CD45^{dim}/CD34⁺/KDR⁺ endothelial progenitors.

DISCUSSION

Despite the strong association between EPC levels and CV outcomes in the general population, we did not find a difference in EPC levels between HIV-infected and uninfected men in this case-control study. Furthermore, EPC levels did not appear to be associated with disease parameters, such as CD4 cell count or viral load.

To our knowledge, only 2 studies have been published examining the link between EPC levels and HIV infection. Teofili et al [10] examined 14 antiretroviral therapy-naive HIV-infected men and women and 15 HIV-uninfected control subjects. They demonstrated that colony-forming unit endothelial cell (CFU-EC) progenitors, but not endothelial colony-forming cell (ECFC) progenitors, were markedly reduced in HIV-infected patients and that proviral HIV DNA was detectable only in CFU-ECs but not in ECFCs. There are many differences between our study and that of Teofili and colleagues that probably explain the divergent results. First, only cell culture techniques were performed in their study, whereas we used flow cytometry, thereby not allowing for a direct comparison between results. Furthermore, the authors noted differences only in CFU-ECs but not ECFCs; of interest, CFU-ECs have been shown to not be true EPCs, whereas ECFCs are clonally distinct and retain the ability to form blood vessels [11]. Thus, in their study, Teofili et al [10] did not demonstrate a difference in the population most important to neoangiogenesis—a finding similar to ours. In addition, our populations differed, because we excluded women, to avoid the confounding effects of estrogen, and included only

younger patients, to avoid the potential influence of subclinical atherosclerosis.

The second study involving HIV-infected individuals by da Silva et al [8] examined the relationship between EPCs, defined as mononuclear cells expressing CD34 and KDR and flow-mediated dilatation, a surrogate marker of endothelial dysfunction. Participants were antiretroviral therapy-naive men and women. Of note, in contrast to our study, da Silva and colleagues found that individuals with HIV infection had lower EPC levels than did individuals without HIV infection, a result that probably reflects important differences in techniques. Enumeration of rare cell populations requires controls and standardization of protocols to ensure inter- and intra-laboratory reproducibility [7]. Accordingly, the ISHAGE protocol, as used in the present study, was developed and validated for clinical application and, in turn, has been applied to EPC enumeration [6, 7]. Furthermore, in our assay, we used red blood cell lysis enabling determination of accurate reporting of CD45^{dim}/CD34⁺/KDR⁺ cells per volume of blood. In contrast, da Silva et al [8] isolated mononuclear cells with use of Ficoll gradient centrifugation, which allows for the determination of the proportion of specific cell populations but not the absolute number of cells per volume of blood. Nonetheless, we were able to replicate the analysis performed by da Silva and colleagues and did not observe any differences between the populations.

Although we did not observe a difference in EPC levels, it remains possible that HIV infection is associated with altered functional aspects of EPCs. Diabetic adults, whose risk of CV disease is also increased, have EPCs with decreased adhesive and proliferative properties, attenuating the propensity of their EPCs to incorporate into vascular structures. Similarly, functional properties of EPCs are known to be important clinically, with higher rates of in-stent restenosis and delayed arterial healing being linked to lower adhesiveness [12]. Of note, HIV-infected men have been shown to have higher rates of in-stent restenosis [13]. Thus, although levels of EPCs appear to be unaffected by HIV infection, the effects of HIV infection on EPC qualitative properties remain to be established.

Another possible reason for the lack of difference between groups may relate to the fact that the HIV-infected individuals in our cohort were relatively early in the course of disease, with relatively preserved immune function (mean CD4 cell count, 478 cells/ μ L). Previous studies have demonstrated increased intimal medial thickness with CD4 cell counts ≤ 200 cells/ μ L [14], and findings of various studies have suggested an association between lower CD4 cell counts and higher viral loads and incidence of CV events [15]. It therefore remains possible that decreased EPC levels and, thus, increased risk of CV disease are not observed until there is a significant decrease in CD4 cell count.

In conclusion, EPC levels were not observed to be significantly reduced in HIV-infected HAART-naive men with relatively preserved immune function. The increased risk of CV disease associated with HIV infection therefore cannot be attributed to reductions in EPC levels on the basis of our data. Because current smokers, individuals with established CV risk factors, and individuals receiving HAART were excluded, this conclusion applies to HIV-infected individuals who are HAART-naive and free of established CV risk factors.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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