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Reduced CD14 expression on classical monocytes and vascular endothelial adhesion markers independently associate with carotid artery intima media thickness in chronically HIV-1 infected adults on virologically suppressive anti-retroviral therapy

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ABSTRACT

HIV infection causes systemic immune inflammation, and increases the risk for cardiovascular (CVD) disease even among those on virologically suppressive anti-retroviral treatment (ART). We performed a biostatistical analysis and screen of candidate cellular and plasma biomarkers for association with carotid artery intima-media thickness (CIMT), independent of traditional CVD risk factors such as age, gender, systolic blood pressure (SBP), lipid levels, smoking and diabetes. We conducted a multi-stage analysis based on a cross-sectional study of CVD risk in HIV-infected subjects age >45 years on ART for >6 months. The goal of this analysis was to identify candidate cellular and plasma biomarkers of CIMT in HIV-1 infected adults. We further sought to determine if these candidate biomarkers were independent of traditional CVD risk factors previously identified in HIV negative adults. High-resolution B-mode ultrasound images of the right common carotid common artery (CCA) were obtained. Plasma soluble inflammatory mediators, cytokines and chemokines were detected. Monocytes were defined by CD14/CD16 expression, and CD8+ T-cell activation by CD38/HLA-DR expression. Subjects were a median of 49.5 years old, 87% male, had a CIMT of 0.73 mm, FRS of 6%, a median viral load of 48 copies/mL, and CD4+ T cell count of 479 cells/μL. Soluble VCAM-1, and expansion of CD14dimCD16− monocytes each associated with higher CIMT independently of age and SBP. These factors are distinct components of a shared atherogenic process; 1) vascular endothelial molecular expression and 2) vascular monocytes that enter into the vascular endothelium and promote atherosclerotic plaque.

1. Introduction

Although HIV-infected individuals are living longer as a result of treatment with effective anti-retroviral therapy (ART), these individuals remain at increased risk of morbidity and mortality due to non-AIDS related diseases associated with aging, such as non-AIDS related cancers and osteoporosis. HIV-infected individuals also have an increased risk of cardiovascular disease [1,2]. Research on cardiovascular disease in HIV [3] has focused on direct atherosclerosis measures, such as carotid intima-media thickness (CIMT) as a predictor of clinical cardiovascular events [4] as HIV-infected individuals tend to be relatively young and at low short-term

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CVD risk. Hsue et al. found that HIV-infected individuals had a greater incidence of plaque in the common and internal carotid artery regions and a higher rate of plaque progression compared to HIV-seronegative controls [5]. HIV drives added CVD risk, although the mechanisms by which this effect is conferred, and effective biomarkers of this process, remain unresolved.

In this manuscript, we examined a panel of candidate biomarkers, both soluble inflammatory mediators and circulating monocyte populations that may mediate inflammation, for their association with CIMT. We conducted a biostatistical modeling analysis based on a cross-sectional study of CVD risk in HIV-infected subjects age >45 years on ART for >6 months. The goal of these models was to identify candidate cellular and plasma biomarkers of CIMT in HIV-1 infected adults. We further sought to determine if these candidate biomarkers were independent of traditional CVD risk factors previously identified in HIV negative adults. We performed this study within the Hawaii Center for AIDS cohort study of older, chronically HIV-1 infected adults with risk factors for CVD on stable virologically suppressive ART. We found evidence for a role for both endothelial cellular adhesion marker expression and altered monocyte phenotypes with higher CIMT in our cohort.

In HIV negative adults, the Framingham risk score (FRS) is employed to estimate 10 year risk for CVD. Over time the FRS system has been extended to encompass several predictive models, each based on distinct outcomes. We wished to determine whether the FRS, and its components, traditional CVD risk factors of age, systolic blood pressure (SBP), gender, current smoking, diabetes and dyslipidemias, associated with CIMT in HIV infected adults. To this end, we selected the FRS Adult Treatment Panel III (ATPIII) [6] that was formulated to predict 'hard' CVD outcomes, such as major cardiac events. However we posit that it is likely that HIV drives additional atherosclerosis mechanisms not captured by the FRS system that are involved in the observed elevated risk for CVD in chronic HIV infection.

It has been hypothesized that HIV-infected individuals have increased risk of CVD through several potential mechanisms including chronic immune activation and inflammation secondary to HIV-induced microbial translocation [7] and low-grade endotoxemia, the direct effects of HIV and viral proteins on macrophage cholesterol metabolism [8–10], and dyslipidemia [11,12] related to specific antiretroviral therapies. Prior reports have suggested that higher CD8+ T cell activation level [13] associates with arterial stiffness, and CMV T cell responses [14] associate with higher CIMT in chronically HIV infected adults. Monocytes, an arm of the innate immune response, are believed to play a critical role in HIV disease and atherogenesis [15]. Inflammatory monocytes transmigrate into the arterial wall and are crucial promoters of atherogenesis, a process that may be accelerated by HIV infection. Peripheral blood monocytes have been divided into subpopulations by their CD14 and CD16 expression [16,17]: 'classical' CD14+CD16− monocytes, 'intermediate' CD14+CD16++ monocytes, and 'non-classical' $CD14^{dim/-}$CD16++ monocytes. Soluble CD14 has been associated with poor HIV disease outcomes [18]. In this report we included a fourth population of cells within the monocyte gate for consideration, a population of $CD14^{dim}$CD16− monocytes. These are cells commonly observed in studies of monocytes, appear to emerge from the classical monocyte gate, but with reduced CD14 expression. CD14 forms a complex with TLR4 that detects lipopolysaccharide (LPS), a factor that drives immune activation in HIV-1 disease [7]. The dynamics of CD14 expression on monocytes may prove informative in HIV and inflammation studies in CVD research.

Inflammatory mediators in the plasma are markers of systemic inflammation. These soluble inflammatory mediators, found in the plasma, may be biomarkers of CIMT in adults with HIV infection. We characterized plasma soluble inflammatory mediators (such as soluble CD14, C-RP, IL-6, TNF, SAA, MCP-1 and others), alongside the relative proportions of the monocyte subsets discussed above, for their relationships to carotid artery intima media thickness (CIMT), and further determined if these factors associated with CIMT after adjustment for component of the FRS score. The purpose of our study was to identify candidate monocyte or plasma biomarkers that may associate with CIMT measurements independent of traditional CVD risk factors.

2. Methods

This is a cross-sectional examination of baseline data from the Hawaii Aging with HIV Cardiovascular Study cohort, a 5-year longitudinal natural history cohort study designed to investigate the role of oxidative stress and inflammation in the pathogenesis of CVD in HIV-infected individuals. Details on enrollment and clinical characteristics are published elsewhere [19]. Briefly, 158 HIV-infected individuals ≥40 years of age were on stable combination antiretroviral therapy (cART) for ≥6 months. Routine HIV and CVD medical histories were obtained. A consensus panel of two study physicians (DCC, CMS) identified prevalent CVD through a process of adjudication of study participants' case report forms. Prevalent CVD was defined as a history of myocardial infarction, angina related to coronary heart disease, coronary artery bypass graft, coronary angioplasty or stent to treat coronary heart disease, ischemic stroke or peripheral vascular disease. Medical assessments obtained included vital signs, plasma HIV RNA, CD4+ T cell count, and lipid profile and glucose after a 12-hour fast, and a 2-hour oral glucose tolerance test (OGTT). Blood pressure was obtained in triplicate after 5 min of resting in triplicate and averaged. Fasting lipid profile included total cholesterol, high-density lipoprotein (HDL-C), directly measured low-density lipoprotein (LDL-C) cholesterol and total triglycerides by enzymatic, colorimetric assay. Hypertension was defined as self-reported diagnosis, use of an antihypertensive medication or measured systolic blood pressure (SBP) equal to or greater than 140 mm Hg or diastolic blood pressure (DBP) equal to or greater than 90 mm Hg [20] or if the subject reported the diagnosis of hypertension. Diabetes mellitus was defined as the use of diabetes medication, or a fasting plasma glucose greater than 126 mg/dL or 2-hour glucose greater than 200 mg/dL on OGTT [21], or if the subject reported the diagnosis of diabetes. Direct vascular measures including CIMT were also obtained. The Committee on Human Studies at the University of Hawaii approved the protocol. Written informed consent was obtained from participants. Participants agreed to have their blood and urine specimens banked and utilized for future research related to HIV and/or cardiovascular health. Subjects were eligible for this analysis if they had completed a CIMT measurement, had calculable Framingham risk scores, and adequate volumes of plasma and viably preserved peripheral blood mononuclear cells (PBMCs).

Framingham risk score (FRS Adult Treatment Panel III (ATPIII)) was calculated based on a model comprised of age, gender, total cholesterol, HDL cholesterol, SBP, treatment of hypertension, and any cigarette smoking in the past month as previously described [6]. FRS was used to categorize subjects into a Framingham Risk Class (FRC) defined as "low" (<10% 10-year risk of CVD), "intermediate" (10–19% risk of CVD), and "high" risk (>20% risk of CVD). Participants with diagnosis of diabetes or adjudicated to have evidence of CVD were automatically classified into the "high risk" (>20% risk of CVD) group.

Immuno-assays: Plasma samples were assayed for soluble inflammatory mediators using antibody coated beads in a high-sensitivity Milliplex assay (Human CVD panels A, B and C, EMD Millipore, Billerica, MA). Standard curves and samples were tested in