Published in final edited form as:

AIDS. 2009 June 1; 23(9): 1059–1067. doi:10.1097/QAD.0b013e32832b514b.

Role of Viral Replication, Antiretroviral Therapy, and Immunodeficiency in HIV- Associated Atherosclerosis

Priscilla Y. Hsue, MD¹, Peter W. Hunt, MD², Amanda Schnell, BA¹, S. Craig Kalapus, RDMS¹, Rebecca Hoh, RD², Peter Ganz, MD¹, Jeffrey N. Martin, MD, MPH^{2,3}, and Steven G. Deeks, MD²

¹ Divisions of Cardiology, University of California, San Francisco

² Positive Health Program of the Department of Medicine, San Francisco General Hospital, University of California, San Francisco

³ Department of Epidemiology and Biostatistics, University of California, San Francisco

Abstract

Objective—HIV-seropositive patients are at higher risk for atherosclerosis than HIV-seronegative persons. This has been variably attributed to antiretroviral drug toxicity, immunodeficiency, and/or HIV-associated inflammation. To evaluate the contributions of these factors to HIV-associated atherosclerosis, we assessed carotid artery intima-media thickness (IMT) in a diverse cohort of HIV-seronegative and seropositive adults, including a unique group of HIV-infected patients who were untreated, had undetectable viral loads and had preserved CD4+ T cell counts (HIV controllers).

Methods and Results—Carotid IMT was measured in 494 subjects, including 33 HIV controllers and 93 HIV-seronegative controls. HIV controllers had higher IMT than seronegative controls even after adjustment for traditional risk factors ($p=0.003$). IMT in controllers was similar to antiretroviral-untreated patients with detectable viremia. Across all subjects, IMT was strongly associated with the presence of HIV disease rather than viral load or CD4+ T cell count. C-reactive protein was higher in HIV controllers than HIV-seronegative persons. Antiretroviral drug exposure was also associated with higher IMT.

Conclusions—Increased atherosclerosis with HIV infection can occur in the absence of antiretroviral therapy, detectable viremia, or overt immunodeficiency. Chronic inflammation—which is higher in controllers than in HIV-uninfected persons—may account for early atherosclerosis in these patients.

INTRODUCTION

The number of people aged 50 and older living with HIV in the U.S. has increased 77% from 2001 to 2005.[1] As the HIV-infected population continues to age, cardiovascular disease will become an increasingly important issue. This is particularly true as emerging data indicate that even after controlling for traditional risk factors (including age), HIV-infected patients have higher rates of atherosclerosis than HIV-seronegative persons.[2,3] Several factors may

Address for correspondence: Priscilla Y. Hsue, MD, Room 5G1, Division of Cardiology, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, CA 94110. Telephone: (415) 206-8257; Fax (415) 206-5100; e-mail: phsue@medsfgh.ucsf.edu.

Presented in part at the 15th Conference on Retroviruses and Opportunistic Infections, Boston, MA February 3-6, 2008 and at the 57th Annual Scientific Session of the American College of Cardiology, Chicago, IL March 29-April 1, 2008

Disclosure

None

contribute to this risk, including direct antiretroviral drug toxicity.[4,5] Other possible contributing factors include HIV-associated inflammation and/or immunodeficiency, as well as direct effects of HIV replication on the endothelium. In a large randomized clinical study comparing continuous antiretroviral therapy to intermittent antiretroviral therapy (the SMART study), interrupting therapy (or not starting therapy) was associated with a higher risk of cardiovascular events,[6] suggesting that any of these HIV-associated factors (viral replication, immunodeficiency, inflammation) may be causally associated with premature cardiovascular disease.

Chronic inflammation and immune activation are now recognized as major risk factors for atherosclerosis.[7] Since the immune system is chronically activated in untreated and to a lesser degree treated HIV disease,[8] we recently postulated that T cell activation is associated with atherosclerosis. Although T cell activation assessed broadly did not correlate with carotid artery intima-media thickness (IMT), a validated measure of atherosclerosis, we found that cytomegalovirus-specific CD8+ T cell responses—which are greatly augmented in HIV disease—were strongly predictive of IMT.[9] These data provided support for an inflammatory/immune component as a mechanism for the accelerated atherosclerosis in HIV-infected individuals. Consistent with this theory, untreated HIV infection has been associated with increased levels of interleukin-6, a pro-inflammatory cytokine and a stimulus to hepatic C-reactive protein production. Higher levels of interleukin-6 strongly predict cardiovascular events and overall mortality in antiretroviral-untreated and treated HIV infection.[10] Considered together, these observations suggest that HIV disease drives premature heart disease via its impact on the immune system and ultimately inflammation. Whether this immunomodulatory and proinflammatory effect is mediated via the level of HIV viremia, the degree of CD4+ cell depletion or other processes remains unclear.

There exists among the HIV-infected population a rare group of individuals who are able to maintain undetectable plasma HIV RNA levels in the absence of any antiretroviral therapy. These “elite” controllers are of high interest scientifically, as one of the primary objectives of a preventative HIV vaccine is to alter the host/virus interaction so that viremia remains low in those who become infected.[11,12] Although the mechanism of virus control in these individuals remains to be defined, it is clear that a strong and persistent HIV-specific immune response exists among these patients.[13] Indeed, we have demonstrated that the level of chronic immune activation is elevated among elite controllers as compared to HIV negatives.[14] Controllers are enriched for a number of genetic polymorphisms that affect the inflammatory response.[15,16]

In order to test the importance of antiretroviral therapy, viral replication, immunodeficiency, and inflammation in driving atherosclerosis, we assessed carotid IMT, a measure of atherosclerosis, and C-reactive protein, a measure of systemic inflammation, in a diverse group of HIV-uninfected and infected adults. Because the HIV controllers lack both exposure to antiretroviral therapy and have neither measurable viremia nor overt immunodeficiency, we focused on these subjects to determine if they are prone to premature atherosclerosis and if so, whether HIV-associated inflammatory responses might be contributory. Accordingly, these HIV controllers were compared to four relevant comparator groups: HIV-seronegative adults, untreated HIV-seropositive adults with detectable levels of viral replication and HIV-seropositive adults receiving long-term antiretroviral therapy both with and without detectable levels of viral replication.

METHODS

Study Subjects

All study participants were recruited from the University of California, San Francisco SCOPE Cohort. By design, nested within this cohort is a relatively large group of rare individuals who were recruited based on their ability to control HIV replication in the absence of therapy. Individuals are referred to SCOPE and this sub-study independently of their cardiovascular risk. All individuals underwent a standard interview and had high resolution ultrasound measurement of carotid IMT. For the purposes of the current analysis, subjects were classified based on their HIV disease and treatment status at the time of the ultrasound studies into four groups: (1) HIV controllers: positive for HIV by standard antibody serological determinations with undetectable HIV RNA level (< 75 copies RNA/mL) in absence of therapy, (2) HIV non-controllers: detectable HIV RNA levels in absence of therapy, (3) highly active anti-retroviral therapy (HAART) responders: on combination antiretroviral therapy with undetectable HIV RNA levels, and (4) HAART non-responders: on combination therapy with persistently detectable HIV RNA levels. HIV-seronegative participants were selected mainly from subjects answering advertisements to participate in research studies who were similar in age and gender to the HIV-infected participants. The University of California, San Francisco Committee on Human Research approved the study, and all patients provided written informed consent.

Risk Factor Assessment

All patients underwent a detailed interview and structured questionnaire given by study investigators covering socio-demographic characteristics, HIV disease history, other co-morbid conditions, health-related behaviors, medication exposure, and family history. Detailed chart review was performed on each patient to carefully ascertain duration of antiretroviral medication. Total and HDL cholesterol, triglycerides, high-sensitivity C-reactive protein (hs-CRP, Dade Behring), and glucose were measured from blood obtained in the fasting state. LDL cholesterol was calculated except in hypertriglyceridemic patients (triglycerides > 400 mg/dl) where it was measured directly.

Carotid IMT Measurements

Carotid IMT was measured by high resolution ultrasound with the GE VividSeven Imaging System and a 10MHz linear array probe, as described previously.[2] Briefly, carotid IMT was measured in 12 predefined segments (6 segments per side) using the standardized protocol of the Atherosclerosis Risk in Communities (ARIC) Study which includes measurements of the near and far wall of the common carotid, the carotid bifurcation and the internal carotid.[17–19] Mean value of 12 segments was calculated. All scans were performed and measurements obtained on digital images using manual calipers by a single experienced vascular technician who was blinded to the subject's HIV status and treatment status. Fifteen patients underwent a second carotid scan within 1 month of the first. The correlation coefficient for the comparison of blinded measurements was > 0.90 .

Statistical Analyses

Unadjusted comparisons between groups were made with Kruskal-Wallis tests followed by pairwise Wilcoxon ranksum tests for continuous variables and chi-square and Fisher's exact tests for categorical variables. Correlations between continuous variables were assessed with Spearman's rank correlation coefficients. Adjusted differences between groups were assessed with linear regression, transforming continuous variables and calculating standard errors with heteroskedasticity-consistent covariance matrix estimators when necessary to satisfy model assumptions.[20] All traditional cardiac risk factors (age, gender, family history, hypertension, fasting serum glucose, smoking (in pack years), and low-density lipoprotein level) were

considered as potential confounders in multivariable models. In addition, we explored the relationship between other potential predictors of IMT as possible confounders including current use of lipid-lowering therapy, mean arterial blood pressure, and fasting serum triglycerides, and HDL on the day of evaluation, considering each of these factors in multivariable models if they were associated with IMT in unadjusted models at the $p < 0.10$ level. All traditional risk factors as well as those additional factors associated with IMT in unadjusted analyses were initially included in multivariable models, then removed in a stepwise manner if their inclusion changed the beta coefficient of the primary predictor (controllers vs. control group) by $< 10\%$. Since hs-CRP may be a mediator of HIV-associated atherosclerosis rather than a true confounder, we assessed models with and without hs-CRP.

RESULTS

Participant Characteristics

The characteristics of the 401 HIV-seropositive participants, including 33 HIV controllers, as well as 93 HIV-seronegative controls are shown in Table 1. Most of the study subjects were men (87% in the HIV-infected group and 77% of controls, respectively) and approximately 50% were Caucasian. The HIV-seropositive subjects were older than the controls (48 years compared to 43 years) and more likely to have smoked in the past. The HIV-seropositive subjects were also more likely to have had a prior history coronary artery disease and a prior history of hypertension, while the controls had a higher LDL cholesterol and HDL cholesterol (Table 1). The median duration of HIV diagnosis ranged from 11 to 15 years, and was similar in each of the four HIV-seropositive groups (Table 1). The median nadir CD4+ cell count for HIV controllers, non-controllers, HAART responders and HAART non-responders was 491, 361, 110, and 70 cells/mm³, respectively. Most of the antiretroviral-treated subjects had received protease inhibitors for part or all of their treatment course. The median duration of HAART was 5.8 years in the responders and 4.9 years in non-responders.

Traditional Risk Factors in HIV-Associated Atherosclerosis

As has been reported by our group and others in the past, HIV-seropositive participants had much higher median IMT than HIV-seronegative participants (0.91mm vs. 0.72mm, $p < 0.001$; see Figure 1). Among all participants, increasing age, cigarette pack years, LDL cholesterol, triglycerides, current lipid lowering therapy use, glucose (but not diabetes), hypertension, and hsCRP were each associated with higher IMT. However, the difference in the carotid IMT between the HIV-seropositive and seronegative individuals remained highly significant even after adjusting for all these risk factors, as well as family history and gender ($p < 0.001$).

Carotid IMT is Increased in HIV Controllers

Despite having no exposure to antiretroviral therapy, no detectable HIV RNA levels using conventional assays, and high CD4+ T cell counts, the HIV controllers had a higher median IMT than the HIV-seronegative subjects (0.91 vs 0.72 mm, $p < 0.001$). This difference remained significant when stratifying by smoking, hypertension, or age (Figure 2). The difference also remained significant when restricting the analysis to those with a current CD4+ cell count > 500 cells/mm³ (Figure 2). The HIV controllers also had higher adjusted mean IMT than HIV-uninfected participants after controlling for traditional risk factors in a multivariable linear regression analysis ($p = 0.005$, Table 2). This difference remained significant even when restricting the multivariable model to those without hypertension and to those with CD4 counts > 500 cells/mm³ ($p = 0.031$).

CD4+ Cell Counts, Plasma HIV RNA levels, and Carotid IMT

The HIV controllers also had a trend toward higher median IMT than HIV-infected non-controllers (0.91 vs. 0.83 mm, $p=0.13$, Figure 1), suggesting that high levels of HIV replication and/or immunodeficiency evidenced by reduced CD4+ counts are not a prerequisite for the development of atherosclerosis in the setting of untreated HIV infection. In fact, higher plasma HIV RNA levels were weakly associated with lower IMT among the HIV non-controllers ($\rho=-0.23$, $p=0.022$), though this relationship was no longer significant after adjustment for age ($p=0.69$). In addition, there was no evidence for a difference in IMT between HIV controllers and either HAART responders ($p=0.49$) or nonresponders ($p=0.86$). There was also no evidence for a relationship between IMT and CD4+ T cell count among HIV controllers ($\rho=0.23$, $p=0.20$) or untreated non-controllers ($\rho=0.13$, $p=0.20$). Finally, there was also no evidence for a relationship between IMT and self-reported nadir CD4+ T cell count among treated HIV-infected participants.

Antiretroviral Treatment and Carotid IMT

Antiretroviral treated patients had a higher median IMT than the untreated patients (0.94 vs. 0.85 mm, $p=0.006$, Figure 3). Furthermore, among all HIV-infected participants, increasing duration of HAART ($\rho=0.20$, $p<0.001$), protease inhibitor use ($\rho=0.19$, $p<0.001$), and nucleoside analogue use ($\rho=0.23$, $p<0.001$) were each associated with thicker IMT. These relationships remained significant after adjustment for traditional cardiac risk factors and the duration of HIV diagnosis ($p\leq 0.024$ for all).

C-Reactive Protein is Increased in HIV Controllers

hsCRP values were significantly higher among all HIV-seropositive patients compared to HIV-seronegative controls (median value of 2 mg/dL in HIV-seropositive versus 1.1 mg/dL in HIV-seronegative, $p<0.001$; see Table 1). This was true even after correcting for traditional risk factors ($p=0.003$). Notably, hsCRP in the HIV controllers was similar to each of the other HIV-infected groups (Kruskal-Wallis test, $p=0.85$). Although CRP was elevated in our controllers, it did not appear to explain the difference in IMT between the controllers and the HIV-seronegative persons since inclusion of hsCRP in the multivariable model changed the adjusted difference between HIV controllers and HIV-seronegative persons by less than 10% and the difference between controllers and HIV-seronegatives remained significant ($p=0.003$).

DISCUSSION

HIV-infected patients are at an increased risk of developing atherosclerosis and cardiovascular disease than age-matched HIV-seronegative adults, yet the mechanisms accounting for this effect remain poorly defined. To clarify the role of several factors putatively associated with atherosclerosis in HIV patients, we used high resolution ultrasound to quantify subclinical atherosclerosis in a large cohort of HIV-infected individuals. Nested within this cohort was a sizeable group of HIV-seropositive individuals who are able to maintain an undetectable HIV viral load in the absence of anti-retroviral therapy (elite controllers). Compared to uninfected controls, carotid IMT was higher among all groups of HIV subjects, irrespective of antiretroviral treatment or the level of viremia. HIV controllers were of particular interest as they lacked the previously suggested key risk factors needed for the development of HIV-associated atherosclerosis namely, exposure to anti-retroviral therapy, high level viremia, and/or advanced immunodeficiency. The unexpected finding of a high IMT in HIV controllers suggests that other factors contribute to the pathogenesis of HIV-associated atherosclerosis. We have previously reported that "elite" control of HIV is typically associated with high levels of T cell activation (as compared to HIV-seronegatives), [9] and extend these observations to include C-reactive protein, a marker of systemic inflammation. Collectively, these data argue

for a possible role of persistent HIV-associated inflammation as a potential cause for accelerated atherosclerosis in HIV disease.

One potential explanation for our findings was that we inadvertently selected a group of HIV-seronegative controls who lacked risk factors and/or had unusually low levels of IMT. This is unlikely as our seronegative controls had carotid IMT values between the 50th and 75th percentile of the Multi-Ethnic Study of Atherosclerosis Study cohort aged 45 to 54 years while our HIV controllers had IMT values far in excess of 75th percentile of this cohort.[21] In fact, the IMT of our HIV-infected subjects, including that of the HIV controllers, was similar to that of patients with heterozygous familial hypercholesterolemia before the era of intensive statin use,[22] a condition almost invariably associated with premature atherosclerosis and coronary heart disease. Our findings suggest that HIV may be associated with a similarly accelerated form of atherosclerosis.

Although “elite” controllers lack readily detectable HIV RNA using conventional assays, they clearly harbor replication competent virus and almost invariably have measurable plasma HIV RNA using very sensitive assays.[23,24] Determining the degree to which this low level HIV replication among HIV controllers is responsible for increased cardiovascular risk has implications for antiretroviral treated patients, since they too often have evidence of persistent low-level viremia (and perhaps low level viral replication) [25–27].

Among HIV controllers, C-reactive protein levels, a marker of systemic inflammation, are elevated and indistinguishable from that of the other HIV-seropositive groups. In uninfected patients, atherosclerosis is a disease with a strong pro-inflammatory component.[7] Activated T cells, macrophages, and mast cells act in concert to release factors leading to atherosclerosis initiation, progression, plaque instability, and intraluminal thrombus formation.[7] Within the context of HIV infection, heightened antigen-specific T cells responses and more recently levels of interleukin-6, a key stimulus to the hepatic production of C-reactive protein, have been associated with accelerated atherosclerotic disease.[9,10] Here, we used measurements of hsCRP to confirm that HIV disease is a pro-inflammatory state, even among the HIV controllers. In uninfected individuals, hsCRP independently predicts cardiovascular disease risk[28,29], while in HIV-seropositive patients, hsCRP clearly predicts mortality.[30] Although the presence of relatively high hsCRP levels in our controllers confirms that long-term host-mediated control of HIV is a pro-inflammatory state, the fact that CRP only explained a small proportion of the differences in IMT between the HIV controllers and HIV-seronegative persons suggests that other inflammatory mediators are more likely causally associated with premature atherosclerosis in HIV disease.

Several factors may contribute to systemic inflammation in HIV-infected controllers. First, HIV infection is associated with an early and rapid destruction of CD4+ T cell population within the intestinal lymphoid tissue.[31] This loss of anti-microbial defense mechanism may lead to the chronic entry of bacterial pro-inflammatory products including endotoxin (lipopolysaccharide) into the blood stream (bacterial translocation).[32] We have demonstrated that compared to controls, HIV controllers (as well as HIV non-controllers) have much higher circulating levels of lipopolysaccharide[14] a product associated with endothelial dysfunction [33,34] and early atherogenesis.[35]. Increases in lipopolysaccharide also activate the innate and adaptive immune systems.[32] Accordingly, higher levels of bacterial lipopolysaccharide potentially result in endothelial dysfunction, immune activation, and chronic inflammation leading to accelerated atherosclerosis among individuals with HIV, including HIV controllers. Second, other viruses in patients with HIV may be involved in immune activation and inflammation and hence atherosclerosis. Most HIV patients, including controllers, are seropositive for cytomegalovirus (CMV). Among all HIV patients, we have shown that increases in CMV-specific T-cell responsiveness correlate directly with the extent of

atherosclerosis.[9] In preliminary data from our group, HIV controllers appear to have higher CMV-specific T cell responses compared to uninfected controls (Hunt, PH unpublished data), suggesting that inflammatory responses related to CMV infection may play a role in HIV-associated atherosclerosis. Lastly, strong HIV-specific T cell responses – the very responses that are thought to help controllers maintain control of HIV replication –[13,36–41] may also be contributing to generalized inflammation even in the absence of clinically detectable viremia.[42]

This study has several limitations that deserve comment. This was a cross-sectional study, where the rate of progression in carotid IMT cannot be measured. This study design makes it particularly challenging to compare treated and untreated disease, as those on and off therapy almost certainly have very different prior disease histories that are difficult to measure. The comparisons between the HIV-seronegative subjects and the HIV controllers is less problematic, as the two groups likely differ primarily in the exposure variable of interest. Also, the magnitude of the differences between the HIV-seronegatives and the controllers is so great that unmeasured confounders are unlikely to solely account for our observed differences. Another potential limitation is the absence of studies correlating carotid IMT with cardiovascular event rates in HIV population. However, extensive data[21] suggest that carotid IMT is a powerful predictor of cardiovascular outcomes in patients without HIV. Extrapolation to HIV patients is reasonable, although remains to be proven. Finally, it is important to stress that the role of inflammation in causing the relatively high levels of IMT in our controllers is based largely on inferences from our prior studies in which we demonstrate that long-term host-mediated control of HIV replication is associated with high levels of T cell activation. We plan in future studies to investigate in more detail the relationship between inflammation and IMT progression among a larger number of HIV controllers. Larger studies will also allow us to control for other potential factors that might be related to persistent inflammation in HIV-infected controllers, including injection drug abuse and/or the presence of certain other co-infections (e.g., CMV, HBV, HCV).

In summary, HIV infection is associated with premature atherosclerosis, as measured by IMT. This occurs even in the absence of detectable viremia, overt immunodeficiency and exposure to antiretroviral therapy, and appears to be independent of traditional cardiac risk factors. While antiretroviral therapy and advanced immunodeficiency likely contribute independently to atherosclerosis in HIV patients, our results with the controllers demonstrate that these elements are not a prerequisite for higher levels of subclinical atherosclerosis. Whether the premature atherosclerosis observed in our controllers is due directly to an inflammatory process remains to be established. From a clinical perspective, our observations suggest that all HIV-infected individuals even those doing apparently well with or without antiretroviral treatment may benefit from aggressive cardiovascular risk management and perhaps the use of anti-inflammatory agents such as statins [43] although this remains unproven treatment in this setting. Finally our observations have implications for individuals treated adequately with antiretroviral therapy by current standards, since it remains possible that persistent low level viral replication which can occur despite otherwise effective therapy may still prove to be harmful. In the future, achieving and maintaining even lower viral loads than current therapies permit in individuals with HIV will need to be investigated both in terms of HIV disease and cardiovascular risk.

Acknowledgments

Sources of Funding

Dr. Hsue is a recipient of a Clinical Scientist Development Award from the Doris Duke Charitable Foundation, a Grant-in-Aid from the American Heart Association, and a grant from the NIH (K23A1066885). This work was supported in part by the UCSF/Gladstone Center for AIDS Research (P30 AI27763, P30 MH59037), NIAID

(AI055273, AI44595, K23AI065244, K24AI069994), the Center for AIDS Prevention Studies (P30 MH62246), the UCSF Clinical and Translational Science Institute (UL1 RR024131-01) and American Foundation for AIDS Research (106710-40-RGRL).

References

1. [Accessed on May 22]. www.cdc.gov
2. Hsue PY, Lo JC, Franklin A, Bolger AF, Martin JN, Deeks SG, Waters DD. Progression of Atherosclerosis as Assessed by Carotid Intima-Media Thickness in Patients With HIV Infection. *Circulation*. 2004
3. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased Acute Myocardial Infarction Rates and Cardiovascular Risk Factors Among Patients with HIV Disease. *J Clin Endocrinol Metab*. 2007
4. Friis-Moller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003;349:1993–2003. [PubMed: 14627784]
5. Sabin CA, Worm SW, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet* 2008;371:1417–1426. [PubMed: 18387667]
6. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006;355:2283–2296. [PubMed: 17135583]
7. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685–1695. [PubMed: 15843671]
8. Hunt PW, Martin JN, Sinclair E, Brecht B, Hagos E, Lampiris H, Deeks SG. T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy. *J Infect Dis* 2003;187:1534–1543. [PubMed: 12721933]
9. Hsue PY, Hunt PW, Sinclair E, Brecht B, Franklin A, Killian M, et al. Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. *Aids* 2006;20:2275–2283. [PubMed: 17117013]
10. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med* 2008;5:e203. [PubMed: 18942885]
11. Walker BD, Burton DR. Toward an AIDS vaccine. *Science* 2008;320:760–764. [PubMed: 18467582]
12. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 2007;27:406–416. [PubMed: 17892849]
13. Emu B, Sinclair E, Favre D, Moretto WJ, Hsue P, Hoh R, et al. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. *J Virol* 2005;79:14169–14178. [PubMed: 16254352]
14. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, et al. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis* 2008;197:126–133. [PubMed: 18171295]
15. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, Pereyra F, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 2007;39:733–740. [PubMed: 17496894]
16. Dolan MJ, Kulkarni H, Camargo JF, He W, Smith A, Anaya JM, et al. CCL3L1 and CCR5 influence cell-mediated immunity and affect HIV-AIDS pathogenesis via viral entry-independent mechanisms. *Nat Immunol* 2007;8:1324–1336. [PubMed: 17952079]
17. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997;146:483–494. [PubMed: 9290509]
18. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. *Am J Epidemiol* 1991;134:250–256. [PubMed: 1877584]

19. Wu KK, Folsom AR, Heiss G, Davis CE, Conlan MG, Barnes R. Association of coagulation factors and inhibitors with carotid artery atherosclerosis. Early results of the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1992;2:471–480. [PubMed: 1342298]
20. Davidson, R.; MacKinnon, J. Estimation and Inference in Econometrics. New York: Oxford University Press; 1993.
21. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* 2008;21:93–111. [PubMed: 18261694]quiz 189–190
22. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001;357:577–581. [PubMed: 11558482]
23. Blankson JN, Bailey JR, Thayil S, Yang HC, Lassen K, Lai J, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. *J Virol* 2007;81:2508–2518. [PubMed: 17151109]
24. Hatano H, Delwart EL, Norris PJ, Lee TH, Dunn-Williams J, Hunt PW, et al. Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. *J Virol* 2009;83:329–335. [PubMed: 18945778]
25. Chun TW, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest* 2005;115:3250–3255. [PubMed: 16276421]
26. Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattoni D, Ferrante P, Wong JK. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. *J Virol* 2003;77:11212–11219. [PubMed: 14512569]
27. Ramratnam B, Mittler JE, Zhang L, Boden D, Hurley A, Fang F, et al. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat Med* 2000;6:82–85. [PubMed: 10613829]
28. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–979. [PubMed: 9077376]
29. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557–1565. [PubMed: 12432042]
30. Lau B, Sharrett AR, Kingsley LA, Post W, Palella FJ, Visscher B, Gange SJ. C-reactive protein is a marker for human immunodeficiency virus disease progression. *Arch Intern Med* 2006;166:64–70. [PubMed: 16401812]
31. Mehandru S, Poles MA, Tenner-Racz K, Manuelli V, Jean-Pierre P, Lopez P, et al. Mechanisms of gastrointestinal CD4+ T-cell depletion during acute and early human immunodeficiency virus type 1 infection. *J Virol* 2007;81:599–612. [PubMed: 17065209]
32. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365–1371. [PubMed: 17115046]
33. Bhagat K, Moss R, Collier J, Vallance P. Endothelial “tuning” following a brief exposure to endotoxin: a mechanism to link infection and infarction? *Cardiovasc Res* 1996;32:822–829. [PubMed: 8944812]
34. Bhagat K, Vallance P. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. *Circulation* 1997;96:3042–3047. [PubMed: 9386173]
35. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, Willeit J. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *J Am Coll Cardiol* 1999;34:1975–1981. [PubMed: 10588212]

36. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 2006;107:4781–4789. [PubMed: 16467198]
37. Harari A, Petitpierre S, Vallelian F, Pantaleo G. Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. *Blood* 2004;103:966–972. [PubMed: 12958069]
38. Saez-Cirion A, Lacabartz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S A* 2007;104:6776–6781. [PubMed: 17428922]
39. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, Rathod A, et al. Genetic and Immunologic Heterogeneity among Persons Who Control HIV Infection in the Absence of Therapy. *J Infect Dis* 2008;197:563–571. [PubMed: 18275276]
40. Emu B, Sinclair E, Hatano H, Ferre A, Shacklett B, Martin JN, et al. HLA class I-restricted T-cell responses may contribute to the control of human immunodeficiency virus infection, but such responses are not always necessary for long-term virus control. *J Virol* 2008;82:5398–5407. [PubMed: 18353945]
41. Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, et al. Lytic Granule Loading of CD8(+) T Cells Is Required for HIV-Infected Cell Elimination Associated with Immune Control. *Immunity* 2008;29:1009–1021. [PubMed: 19062316]
42. Hunt, PW.; Sinclair, E.; Hatano, H.; McCune, JM.; Emu, B.; Tan, QX., et al. Relationship between HIV-specific Immune Response, T Cell Activation, and CD4+ T Cell Depletion in HIV-infected Patients with Undetectable Plasma HIV RNA Levels in the Absence of Therapy. Program and Abstracts from the 15th Conference on Retroviruses and Opportunistic Infections, 2008; Boston, MA. 2008. Abstract # 353
43. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med* 2005;352:29–38. [PubMed: 15635110]

ART+: on antiretroviral therapy
 ART-: not on antiretroviral therapy
 VL<75: undetectable HIV RNA
 VL>75: detectable HIV RNA

Figure 1. Comparison of Carotid IMT in HIV-infected and uninfected individuals

Antiretroviral-untreated patients with undetectable viral loads (HIV controllers) had a higher median carotid IMT than the HIV-seronegative persons, even after controlling for traditional risk factors. Carotid IMT was comparable in the HIV controllers and untreated HIV non-controllers.

Figure 2. IMT in HIV Controllers and HIV-seronegatives stratified by potential confounders
Carotid IMT was higher in HIV controllers than HIV-seronegative persons when stratified by age (Panel A), the presence or absence of hypertension (Panel B), and a history of tobacco smoking (Panel C). Carotid IMT was also higher among the controllers than HIV-seronegatives even after restricting the analysis to those with CD4+ T cell counts above 500 cells/mm³ (Panel D).

Figure 3. Effect of HAART on carotid IMT

Carotid IMT was higher in patients who were receiving highly active antiretroviral therapy (HAART) than in either antiretroviral untreated patients or HIV-seronegative persons. The effect of HAART remained significant after controlling for traditional risk factors.

Table 1
Coronary risk factors and laboratory values in HIV-infected and uninfected subjects.

	HIV-negative Controls (n=93)	ART- VL < 75 HIV Controllers (n=33)	ART- VL>75 Non- controllers (n=96)	ART+ VL<75 Responders (n=180)	ART+ VL>75 Non- responders (n=92)
Age (years) ^I	44(38 to 49)	49(44 to 53)	45(38 to 51)	49(43 to 54)	47(42 to 53)
Male/female	72/21	23/10	85/11	159/21	83/9
Caucasian	56(60%)	14(42%)	43(45%)	123(68%)	46(50%)
History of coronary disease	1 (1%)	3(9%)	9(9%)	17(10%)	4(4%)
Coronary risk factors:					
Ever smoker	49 (53%)	28(85%)	71(74%)	106(59%)	59(64%)
Diabetes	2 (2%)	2(6%)	4(4%)	11(6%)	7(8%)
Hypertension	5(5%)	11(33%)	21(22%)	55(31%)	23(25%)
Mean Arterial BP (mm Hg) ^I	90 (85 to 97)	93 (87 to 97)	91 (86 to 97)	91 (86 to 96)	91 (85 to 97)
FH CAD	16(17%)	2(6%)	17(18%)	44(24%)	18(20%)
Laboratory values:					
HDL-cholesterol (mg/dL) ^I	47(42 to 60)	45(35 to 52)	43(35 to 49)	43(36 to 52)	41(33 to 50)
LDL-cholesterol(mg/dL) ^I	112(93 to 142)	102(70 to 127)	97(77 to 118)	108(91 to 130)	98(68 to 119)
Triglycerides (mg/dL) ^I	99(71 to 143)	105(86 to 172)	92(67 to 132)	173(116 to 312)	147(94 to 226)
hs-CRP (mg/L) ^I	1.1(0.4 to 3.1)	2.0(1.0 to 5.0)	1.8(0.8 to 4.8)	2.2(0.7 to 4.9)	1.7(0.85 to 4.75)
Glucose (mg/dL) ^I	85(79 to 90)	89(80 to 101)	87(81 to 94)	89(83 to 100)	84(77 to 91)
Years since HIV diagnosis ^I	-	14 (9 to 19)	11(4 to 16)	15(10 to 18)	13(10 to 17)
Total years of HAART ^I	-	0	0	5.8(3.5 to 8.0)	4.9(3.1 to 7.6)
Current CD4 count ^I	-	765 (556 to 1037)	489 (356 to 667)	489 (311 to 659)	233 (134 to 375)
Nadir CD4 count ^I	-	491(342 to 800)	361(290 to 500)	110(27 to 201)	70(20 to 177)
Plasma HIV RNA level ^I (log ₁₀ copies/ml)	-	<75	3.5 (2.8 to 4.2)	<75	3.8 (3.1 to 4.5)

^I median value shown (IQR)

Table 2
 IMT in HIV Controllers and HIV-uninfected Individuals after Adjustment for
 Traditional Cardiac Risk Factors

Factor	Unadjusted		Adjusted	
	Mean Change in IMT (mm) (95% CI)	P Value	Mean Change in IMT (mm) (95% CI)	P Value
<u>HIV Status</u>				
HIV-uninfected	Reference Group	-	Reference Group	-
HIV Controller	+0.26 (0.13 to 0.39)	<0.001	+0.16 (0.05 to 0.26)	0.005
Age, per 10 year increase	+0.17 (0.10 to 0.21)	<0.001	+0.09 (0.06 to 0.12)	<0.001
Female gender	-0.02 (-0.11 to 0.15)	0.79	-	-
Family history of CAD	-0.05 (-0.16 to 0.05)	0.63	-	-
Glucose, per 10mg/dL increase ¹	+0.09 (-0.002 to 0.18)	0.055	-	-
(Glucose, per 10mg/dL increase) ²	-0.0004 (-0.0008 to 0.0001)	0.058	-	-
Hypertension	+0.34 (0.12 to 0.56)	0.043	+0.16 (-0.02 to 0.35)	0.083
Smoking, per 10 pack-year increase	+0.04 (-0.01 to 0.09)	0.098	+0.02 (-0.02 to 0.05)	0.40
LDL level, per 10 mg/dL increase	+0.006 (-0.004 to 0.017)	0.212	+0.01 (0.001 to 0.02)	0.027
Triglyceride level, per 100mg/dL increase	+0.04 (0.01 to 0.08)	0.008	-	-
Lipid-lowering Therapy Use	+0.33 (0.05 to 0.62)	0.023	-	-

¹ glucose modeled as a quadratic term

² glucose modeled as a squared term