Published in final edited form as:

J Acquir Immune Defic Syndr. 2019 May 01; 81(1): e21–e23. doi:10.1097/QAI.0000000000002001.

PD-1+ and TIGIT+ CD4 T cells are associated with coronary artery calcium progression in HIV-infected treated adults

Scott Bowler, BS[1], **Glen M. Chew, PhD**[1], **Matt Budoff, MD**[2], **Dominic Chow, MD, PhD**[1], **Brooks I. Mitchell, MS**[1], **Michelle L. D'Antoni, PhD**[1], **Chathura Siriwardhana, PhD**[1], **Lishomwa C. Ndhlovu, MD, PhD**[1], and **Cecilia Shikuma, MD**[1]

[1]University of Hawaii, Honolulu, HI USA

[2]Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, California, USA

With the advent of antiretroviral therapy (ART), AIDS-related morbidity and mortality has declined allowing age-related diseases, such as cardiovascular disease (CVD), to emerge as new challenges for this population. With an effect size of approximately 1.5 to 2.0, the impact of HIV on CVD is independent of traditional cardiovascular risk factors and antiretroviral medications [1]. Immune activation of monocytes and macrophages have been implicated in the higher CVD risk in individuals with chronic HIV. Coronary artery calcium (CAC) is an indicator of subclinical coronary artery atherosclerosis predictive of coronary events including the onset of myocardial infarction and coronary related deaths [2]. Two research groups, including our own, have published that activated $CD16^+$ monocytes/macrophages predict greater CAC progression among HIV-infected persons over a 2 year period [3 4].

HIV-associated immune dysfunction is also characterized by T cell dysfunction; their role in HIV associated atherosclerosis has been less studied. T cell exhaustion is characterized by an expansion of negative checkpoint receptors (NCR) including PD-1 (programmed cell death protein 1), TIM-3 (T cell immunoglobulin and mucin-domain containing-3), and TIGIT (T cell immunoreceptor with Ig and ITIM domains). During chronic infection or cancer, T cell exhaustion results in the progressive loss of effector function, up regulation of inhibitory receptors, and failure to transition to a quiescent state [5]. We have recently reported that the expansion of NCR on $CD4^+$ T cells is associated with co-morbidities of cognitive impairment and fat loss in HIV infected individuals on ART [6 7]. In this study, we sought to examine the impact of NCR-expressing T cells on CVD. We hypothesized that higher baseline PD1- and/or TIGIT-expressing $CD4^+$ T cells will be associated with progression of CAC after 2 years in chronically HIV-infected individuals on stable ART.

T cell immunophenotyping was performed on banked peripheral blood mononuclear cells (PBMC) from HIV-infected individuals enrolled in the Hawaii Aging with HIV-Cardiovascular Disease (HAHC-CVD) Cohort Study [8], a longitudinal study of subclinical

Corresponding Author: Scott Bowler, BS, University of Hawaii, 651 Ilalo St, BSB 231, Honolulu, HI 96813, Phone: (808) 692-1310, Fax: (808) 692-1345, sbowler@hawaii.edu.

CVD risk in individuals with chronic HIV age > 40 years and on ART for ≥ 3 months. Data on CVD risk factors, as well as metabolic data from fasting blood were available and allowed calculation of the Framingham Risk Score (FRS) using the National Cholesterol Education Program website (http://hp2010.nhlbihin.net/atpiii/calculator.asp). As previously reported [8], computer tomography (CT) examinations for CAC were performed locally in Honolulu, HI using a dual source CT scanner (Siemens 64-slice Somatom) with quantification of CAC centrally at the Los Angeles Biomedical Research Institute (M Budoff).

Cryopreserved PBMC were thawed and stained for viability and the frequency of expression at baseline of TIGIT, PD-1, and TIM-3 on $CD4^+$ and $CD8^+$ T cells were assessed by flow cytometry following previously published methods [9]. Isotype controls or fluorescence minus one controls were used to facilitate gating. Software-based compensation was performed on FlowJo (Treestar).

The predictive impact of NCR-expressing T cells and other immunologic parameters on 2-year change in CAC was assessed by logistic regression, dichotomizing the participants into those who demonstrated progression of CAC and those whose level showed no progression. A multivariate logistic regression model was constructed. Because of the small sample size, FRS was utilized as a composite marker of traditional CVD risk factors. We, as well as others, have reported a correlation between monocyte immune activation and change in CAC. We therefore further examined the relationship between NCR-expressing T cells and monocyte subsets based on CD16 expression (classical, intermediate and non-classical monocytes), hypothesizing that any impact of NCR-expressing T cells on CAC may be mediated via an increase in monocyte immune activation. A two-sided p-value (p) < 0.05 was considered as statistically significant. Analyses were performed using SPSS version 24 (IBM, Armonk, NY).

The data consisted of 43 HIV-infected participants who were predominantly male (88%) and Caucasian (62%) with a median age of 52 years, median CD4 count of 518 cells/μL, self-reported median nadir CD4 count of 93.5 cells/μL, median CD4:CD8 T cell ratio of 0.68 at baseline, and 3 (13%) were on a statin. The median duration on ART was 14 years and 83.7% had plasma HIV RNA levels < 50 copies/mL. Those with detectable HIV RNA levels had a median viral load of 414 copies/mL. CAC was present in 51% (n=22) of the population at baseline and 25.5% (n=11) showed an increase in CAC after 2 years. At baseline, the median percent expressions of TIGIT, TIM-3, and PD-1 on $CD4^+$ T cells were 22.6 (IQR 16.4, 28.3), 15.7 (11.0, 20.9), and 36.3 (30.3, 48.3) percent respectively and on $CD8^+$ T cells 44.0 (36.4, 59.4), 21.9 (18.1, 28.0) and 36.3 (25.7, 47.3) percent.

In univariate logistic regression analyses, significant relationships to 2-year change in CAC was seen for various single or dual-expression of NCR on CD4+ T cells and for FRS but not for nadir or current CD4 count at baseline. In multivariate analyses adjusting for FRS, baseline expressions on CD4+ T cells of $PD\text{-}1^+$ and $TIGIT^+$, and dual expression of $PD\text{-}1^+TIGIT^+$ were significantly associated with a 2-year increase in CAC (odds ratio=1.110 to 1.261, all $p<0.05$). Similar results were obtained when analyses were restricted to individuals with undetectable viral loads. Interestingly these associations were restricted to

$CD4^+$ T cells and no association was found between NCR-expressing $CD8^+$ T cells and 2-year change in CAC.

Relationships between NCR-expressing $CD4^+$ T cells and monocyte subsets were examined. $TIGIT^+$ $CD4^+$ T cells approached significance with absolute numbers of intermediate monocytes (rho=0.273 p=0.088). PD-1^+, $TIGIT^+$, and PD-1^+TIGIT^+ $CD4^+$ T cells trended towards significance with absolute numbers of non-classical monocytes (rho=0.302 p=0.058, rho=0.274 p=0.087, rho=0.322 p=0.053, respectively). We found no associations between PD-1 or TIGIT expressing $CD4^+$ T cells and classical monocyte numbers.

Our analyses revealed a possible new and unexpected association between higher frequency of PD-1^+, $TIGIT^+$ and dual PD-1+ TIGIT+-expressing $CD4^+$ T cells and 2-year progression of CAC. This association was specifically seen for $CD4^+$ T cells but not $CD8^+$ T cells. The role of NCR expression on $CD4^+$ in CVD is poorly understood. Much of the general literature suggests that lower, rather than higher, level of NCR on T cells is associated with increased risk of atherosclerosis. In triple ligand knockout mice (PD-$L1^{-/-}$PD-$L2^{-/-}$$Ldlr^{-/-}$), an elevated activated T cell response and increased serum TNF-α level correlated with increased atherosclerosis [10]. In humans, it has been reported that PD-1 is decreased, rather than increased, on $CD4^+$ and $CD8^+$ T cells in coronary artery disease patients which correlated to increased T cell proliferation and increased pro- and decreased anti-inflammatory cytokine secretion [11]. It is possible that the impact of NCR-expressing CD4+ T cells on CAC may be mediated via its relationship to monocyte immune activation. Our analyses revealed only trends towards a relationship, but this may be secondary to the small sample size of our cohort. Alternatively, or in addition, a direct effect of NCR-expressing CD4+ T cells on coronary artery disease is possible. Similar to monocytes, T cells are directed to blood vessel walls. T cells within atherosclerotic lesions produce pro-inflammatory cytokines, such as collagen-inhibiting gamma interferon, which may contribute to the development of coronary artery disease [12].

This study is limited by the small sample size and lack of PD-L1 assessment on corresponding monocytic cells. Furthermore, our study was able to only demonstrate the association specifically with coronary artery calcification. Coronary artery disease in HIV is associated with a marked increase in non-calcified coronary artery plaque burden [13] and this could not be ascertained because CT angiography was not done. However, the strengths of the study include the extensive immunologic characterization of the patient cohort and the longitudinal assessment of CAC burden quantitated by an experienced central reading center.

In conclusion, our study found an association between PD-1 and TIGIT expressing $CD4^+$ T cells singly or in combination and 2-year increase in CAC among individuals with chronic HIV infection on ART. In HIV-infection, PD-1 expression levels are up-regulated on $CD4^+$ T cells and we hypothesize that this may contribute to the increased risk of CVD in this population. More research is needed to confirm these results.

Acknowledgments

Conflicts of Interest and Sources of Funding: Cecilia Shikuma has received grants (R01HL095135 and U54MD007584) from the National Institutes of Health. For the remaining authors, none were declared.

References

1. Norata GD, Caligiuri G, Chavakis T, et al. The Cellular and Molecular Basis of Translational Immunometabolism. Immunity 2015;43(3):421–34 doi: 10.1016/j.immuni.2015.08.023[published Online First: Epub Date]|. [PubMed: 26377896]
2. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. The New England journal of medicine 2008;358(13):1336–45 doi: 10.1056/NEJMoa072100[published Online First: Epub Date]|. [PubMed: 18367736]
3. Baker JV, Hullsiek KH, Singh A, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. Aids 2014;28(6):831–40 doi: 10.1097/QAD.0000000000000145[published Online First: Epub Date]|. [PubMed: 24370480]
4. Zungsontiporn N, Tello RR, Zhang G, et al. Non-Classical Monocytes and Monocyte Chemoattractant Protein-1 (MCP-1) Correlate with Coronary Artery Calcium Progression in Chronically HIV-1 Infected Adults on Stable Antiretroviral Therapy. PloS one 2016;11(2):e0149143 doi: 10.1371/journal.pone.0149143[published Online First: Epub Date]|. [PubMed: 26867220]
5. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nature reviews. Immunology 2015;15(8):486–99 doi: 10.1038/nri3862[published Online First: Epub Date]|.
6. Gangcuangco LM, Chow D, Souza SA, et al. HIV Immune Dysregulation Predicts Lean Tissue and Fat Changes During Chronic Disease. Conference on Retroviruses and Opportunistic Infections Boston, MA, 2018.
7. Mitchell BI, Chew GM, Byron MM, et al. Association between Dual TIM-3 and PD-1 Expressing CD4+ T cells and Neurocognitive Function in Chronic Stable HIV Disease. International AIDS Society Durban, South Africa, 2016.
8. Shikuma CM, Barbour JD, Ndhlovu LC, et al. Plasma monocyte chemoattractant protein-1 and tumor necrosis factor-alpha levels predict the presence of coronary artery calcium in HIV-infected individuals independent of traditional cardiovascular risk factors. AIDS research and human retroviruses 2014;30(2):142–6 doi: 10.1089/AID.2013.0183[published Online First: Epub Date]|. [PubMed: 23984974]
9. Chew GM, Fujita T, Webb GM, et al. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. PLoS pathogens 2016;12(1):e1005349 doi: 10.1371/journal.ppat.1005349[published Online First: Epub Date]|. [PubMed: 26741490]
10. Gotsman I, Sharpe AH, Lichtman AH. T-cell costimulation and coinhibition in atherosclerosis. Circulation research 2008;103(11):1220–31 doi: 10.1161/CIRCRESAHA.108.182428[published Online First: Epub Date]|. [PubMed: 19028921]
11. Lee J, Zhuang Y, Wei X, et al. Contributions of PD-1/PD-L1 pathway to interactions of myeloid DCs with T cells in atherosclerosis. Journal of molecular and cellular cardiology 2009;46(2):169–76 doi: 10.1016/j.yjmcc.2008.10.028[published Online First: Epub Date]|. [PubMed: 19056397]
12. Ilhan F, Kalkanli ST. Atherosclerosis and the role of immune cells. World journal of clinical cases 2015;3(4):345–52 doi: 10.12998/wjcc.v3.i4.345[published Online First: Epub Date]|. [PubMed: 25879006]
13. Post WS, Budoff M, Kingsley L, et al. Associations between HIV infection and subclinical coronary atherosclerosis. Ann Intern Med 2014;160(7):458–67 doi: 10.7326/M13-1754 [PubMed: 24687069]