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Short Communication: Plasma Lymphocyte Activation Gene 3 and Subclinical Coronary Artery Disease in the Multicenter AIDS Cohort Study

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Abstract

Chronic inflammation, including among people with HIV (PWH), elevates immune cell expression of lymphocyte activation gene 3 (LAG3); however, low plasma LAG3 predicts cardiovascular disease (CVD) events in the general population. The associations among LAG3 plasma levels, subclinical atherosclerosis, inflammation, and HIV infection have not been well described. We measured plasma LAG3 in 704 men with and without HIV from the multicenter AIDS cohort study, who underwent coronary computed tomography angiography. HIV serostatus was not independently associated with LAG3 after adjustment for sociodemographic and CVD risk factors. Current smoking status and African American race were associated with lower LAG3, and age and sTNFαRI concentration were associated with greater LAG3. LAG3 was not associated with coronary artery stenosis. Thus, no difference was found in plasma LAG3 concentration by HIV serostatus, and no association between LAG3 and subclinical coronary atherosclerosis in men with and without HIV was observed.

Keywords: LAG3, cardiovascular disease, HIV

People with HIV (PWH) are up to two times more likely to have an acute myocardial infarction compared to people without HIV.[1,2] Both traditional and nontraditional cardiovascular disease (CVD) risk factors, including inflammation, contribute to this risk.[3] Scavenger receptor class B type 1 (SR-B1) protein is a transporter in reverse cholesterol transport, the principal function of high-density lipoprotein (HDL). In men in the multi-ethnic study of atherosclerosis (MESA), a general population cohort, a risk allele of *SCARB1*, the gene encoding SR-B1, was associated with CVD events.[4] This association was mediated by *SCARB1*'s interaction with lymphocyte activation gene 3 (*Lag3*), which encodes the T cell inhibiting protein LAG3. Low plasma LAG3 levels also predicted coronary heart disease events but not common carotid intimal medial thickness or coronary artery calcium (CAC) in the MESA.[5]

Greater expression of immune checkpoint molecules, including LAG3, is seen in systemic inflammatory states, including HIV infection, and is associated with potential adverse consequences for organs.[6] Cell surface LAG3 regulation through cleavage produces plasma LAG3.[5] The relationship between plasma LAG3 and CVD in PWH is not well

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described. HIV infection and chronic inflammation result in greater cell surface LAG3 expression,[7] but less is known about their effects upon plasma LAG3. In the multicenter AIDS cohort study (MACS), we previously found that multiple markers of systemic inflammation were associated with coronary artery stenosis in PWH.[8,9] We hypothesized that lower plasma LAG3 in PWH would be associated with a greater prevalent subclinical coronary atherosclerosis as measured by computed tomography (CT) angiography, which was not used in previous studies examining the association between LAG3 and subclinical atherosclerosis.[5,6] In addition, CAC does not detect noncalcified plaque, which is less stable and more prevalent in PWH.[10]

This study aimed to investigate the cross-sectional associations among the following: (1) plasma LAG3, HIV serostatus, and inflammation and between (2) plasma LAG3, subclinical coronary artery stenosis, and inflammation in the MACS.

The MACS is a prospective cohort of men who have sex with men, including men with HIV (MWH) and men without HIV (MWoH), recruited from four centers. Participation in the MACS includes a semiannual standardized interview, physical examination, and laboratory tests. All MACS participants signed informed consent, and the study was approved by Institutional Review Boards at each site.

This study sample was drawn from the MACS Cardiovascular Ancillary Study, and inclusion criteria were as follows: ages 40–70 years, weight <300 pounds, estimated glomerular filtration rate ≥60 mL/min/1.73 m2, and no history of intravenous contrast allergy, cardiac surgery or percutaneous coronary intervention.[10] Subclinical coronary atherosclerosis was measured by coronary CT angiography (CTA) from 2010 to 2013, as previously described.[11] Images were interpreted by readers masked to participant history.[11]

Serum IL-6, ICAM-1, sCD163, and sCD14 were measured by ELISA (R&D Systems, Minneapolis, MN). sTNFαRI and sTNFαRII were measured by a multiplex assay, and CCL2 was measured using a singleplex assay (Millipore, Billerica, MA). Plasma LAG3 was measured by ELISA (Ray Biotech, Inc., Norcross, GA) as previously described.[5] In models in which LAG3 was the outcome, LAG3 was natural log transformed to fit a normal distribution.

Descriptive comparisons of variables between MWH and MWoH were measured using a Student *t*-test for normally distributed continuous variables, a Wilcoxon rank-sum test for non-normally distributed continuous variables, and a chi-squared test for categorical variables. For separate analyses, the primary outcomes were (1) plasma $\log_e$ LAG3 and (2) presence of moderate to severe coronary artery stenosis (≥50% stenosis) in ≥1 coronary artery segment.

We assessed the correlates of LAG3, and the association between LAG3 and coronary artery stenosis using multivariable linear and logistic regression models, respectively. The covariates, which were added to the multivariable models regardless of outcome or the participants included, included age, race, study center, and HIV serostatus, systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, total cholesterol, HDL cholesterol (HDL-C), lipid-lowering medication use, body mass index, and smoking status (current, former, or never). Levels of the inflammatory and monocyte activation markers, IL-6, ICAM-1, sTNFαRI, sTNFαRII, sCD163, sCD14, and CCL2, standardized to their means, were added individually to multivariable models. In models limited to MWH, we included HIV-related variables: a history of an AIDS-defining malignancy or opportunistic infection, current and nadir $CD4^+$ T cell count, current viral load (VL), and duration of antiretroviral therapy use on LAG3.

The MACS cardiovascular ancillary study included 765 men, among whom 704 (410 MWH and 294 MWoH) had plasma samples from 12 ± 8 months before the CTA. MWH were younger, included more black men, and had lower total cholesterol and HDL-C ($p < .01$, for all). Out of 410 MWH, 363 were on antiretroviral therapy (ART), of whom 70 had a detectable VL. Mean [standard deviation (SD)] current CD4 T cell count in MWH was 624 (271) cells, and mean (SD) nadir CD4 T cell count in MWH was 329 (271) cells. LAG3 was lower in MWH than in MWoH ($p < .01$). However, in the multivariable model, positive HIV serostatus was not associated with LAG3 ($\beta = -0.11$, 95% confidence interval (CI) (–0.29 to 0.08)).

Current smoking status and African American race were associated with lower LAG3, and older age was associated with greater LAG3 (Table 1). In an adjusted model, sTNFαRI was significantly associated with LAG3 ($\beta = 0.10$, 95% CI 0.02–0.20), although other inflammatory markers were not. In a multivariable model limited to MWH, current ART use was associated with lower LAG3 ($p = .04$).

No statistically significant association was noted between LAG3 levels and moderate to severe coronary artery stenosis in a multivariable model (odds ratio = 0.94, 95% CI 0.77–1.15).

Our study is the first published evaluation of associations between plasma LAG3 levels and subclinical atherosclerosis in MWH. Plasma LAG3 was significantly lower in MWH than in MWoH in an unadjusted analysis, but not in a fully adjusted model, and no significant association between LAG3 and moderate to severe coronary artery stenosis was observed. Similarly, Pallikkuth *et al.* found no associations between soluble LAG3 levels and cardiac function, including markers such as cardiac output, or vascular stiffness in a group of 84 study participants (21 healthy controls, 21 PWH on ART, and 42 ART-naive PWH).[6]

Patient characteristics, including age, can affect T cell checkpoint molecule expression. We found that older age was associated with greater LAG3 and that current smoking and black race, compared to non-Hispanic white race, were associated with lower plasma LAG3. We did not find a significant association between lipid medication use and plasma LAG3 levels. Similarly, in the MESA, greater smoking pack years were associated with lower plasma LAG3, but older age was associated with lower plasma LAG3 and black race (compared to non-Hispanic white or Hispanic ancestry) was associated with greater LAG3 levels.[5] The discrepancies may have been secondary to unmeasured differences in the cohorts not accounted for in our models.

Unlike Pallikkuth *et al.* who found that greater soluble LAG3 was detected in ART-naive PWH compared to levels in both PWH on ART and control participants,[6] we did not find that HIV-positive serostatus was associated with plasma LAG3 levels in our cohort in which the majority of MWH were on ART and had undetectable HIV RNA levels.

We did not find an association between plasma LAG3 levels and subclinical coronary atherosclerosis in MWH and MWoH; however, the relationship between LAG3 levels and

Table 1. Associations of Covariates with Plasma LAG3

	Mean LAG3 (pg/mL)	*Unadjusted beta coefficient (95% CI)*	p	*Adjusted beta coefficient (95% CI)*	p
HIV-positive serostatus	3152	−0.38 (−0.56 to −0.20)	<.01	−0.11 (0.29 to 0.07)	.24
HIV-negative serostatus	3835	(reference)		(reference)	
Age (years)				0.02 (0.005 to 0.03	<.01
Race					
Non-Hispanic White				(reference)	
Non-Hispanic Black				−0.50 (−0.72 to −0.28)	<.01
Other				−0.17 (−0.46 to 0.12	.26
Body mass index (kg/m^2)				0.008 (−0.01 to 0.03)	.42
Smoking status					
Never				(reference)	
Former				0.07 (−0.14 to 0.27)	.51
Current				−0.29 (−0.53 to −0.06)	.02
High density lipoprotein cholesterol (mg/dL)				0.004 (−0.002 to 0.01)	.17
Total cholesterol (mg/dL)				0.001 (−0.001 to 0.003)	.36
Lipid-lowering medication use				0.002 (−0.19 to 0.19)	.99
Systolic blood pressure (mmHg)				0.003 (−0.003 to 0.01)	.35
Antihypertension medication use				0.05 (−0.14 to 0.25)	.59
Fasting glucose (mg/dL)				−0.0003 (−0.004 to 0.004)	.87
Diabetes medication use				−0.20 (−0.57 to 0.16)	.28
IL-6				0.07 (−0.02 to 0.15)	.13
ICAM-1				0.02 (−0.07 to 0.10_	.72
sTNFαRI				0.10 (0.02 to 0.20)	.02
sTNFαRII				0.06 (−0.03 to 0.15)	.18
sCD163				0.003 (−0.08 to 0.08)	.94
sCD14				−0.03 (−0.11 to 0.06)	.53
CCL2				0.04 (−0.04 to 0.13)	.32

The fully adjusted model in which LAG3 was the outcome included the following covariates: age, HIV serostatus, race, study center, body mass index, smoking status (never, former, and current), HDL-C, total cholesterol, lipid-lowering medication use, systolic blood pressure, antihypertension medication use, fasting glucose, and diabetes medication use.

In the boxed section of Table, the results of the fully adjusted models to which each inflammatory or monocyte activation marker was added separately are listed. Each inflammatory or monocyte activation marker was standardized to its mean before inclusion to the model.

CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LAG3, lymphocyte activation gene 3.

subclinical atherosclerosis may be different from that between LAG3 and CVD events. Given the effect of HIV infection on cell surface LAG3, plasma LAG3 represents a biomarker that merits further investigation, possibly with respect to clinical CVD events.

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