Short Communication: Effect of Antiretroviral Therapy on Circulating Damage-Associated Molecular Pattern Molecules and CD4 Immune Reconstitution in HIV-Infected Individuals

Daniel R. Drozd,¹ Susan M. Graham,^{1–3} Heidi M. Crane,¹ Joseph A.C. Delaney,³
Mari M. Kitahata,¹ and Wayne Conrad Liles^{1,2}

Abstract

We examined levels of the damage-associated molecular pattern molecules, HMGB1 and S100A9, in individuals for whom stored samples were available before and after antiretroviral therapy (ART) initiation, to determine their association with CD4 reconstitution. Mean HMGB1 levels (1.95 ng/ml vs. 3.02 ng/ml) and the proportion of individuals with detectable S100A9 (19.6% vs. 43.1%) significantly increased after ART. Detectable post-ART S100A9 was independently associated with impaired immune reconstitution.

ALTHOUGH ANTIRETROVIRAL THERAPY (ART) reduces immune activation in HIV-infected individuals, inflammation remains increased in treated HIV infection,¹ likely contributing to HIV pathogenesis,² impaired immune reconstitution,³ and adverse clinical outcomes.⁴ Damage-associated molecular patterns (DAMPs) are endogenous innate immune activation molecules capable of activating the same pattern recognition receptors as exogenous pathogen-associated molecular patterns, such as lipopolysaccharide (LPS).⁵ They are involved in the pathogenesis of a range of inflammatory clinical conditions in HIV-uninfected individuals.⁶ Limited data exist examining DAMPs in HIV infection.⁷

This study was conducted using data and specimens obtained from HIV-infected individuals enrolled in the University of Washington (UW) HIV cohort.⁸ We identified 51 individuals ≥ 18 years of age with plasma specimens available ≤ 1 year before starting initial combination ART regimen and ≥ 2 years after viral suppression [HIV RNA viral load (VL) ≤ 400 copies/ml]. We excluded individuals with any interval VL > 400 copies/ml or who developed any AIDS-defining illness or malignancy.

Linked plasma specimens cryopreserved at -70°C were obtained from the UW Center for AIDS Research (CFAR) specimen repository. Assays were run in a single batch in the Liles laboratory. HMGB1 was measured by enzyme-linked immunosorbent assay (ELISA) from Shino-Test Corporation

(Tokyo, Japan); S100A9 by ELISA from R&D Systems (Minneapolis, MN); and LPS by lymphocyte amebocyte lysate assay from Lonza Corporation (Basel, Switzerland).

To determine whether ART reduces DAMP inflammation, we compared pre- and post-ART HMGB1, S100A9, and LPS levels. Dichotomous measures [detectable vs. below the limit of quantification (BLQ)] were used when $> 50\%$ of results were below the limit of detection of the assay. Non-normally distributed biomarker values were $\log_{10}$ transformed. Values were compared using paired *t*-tests and McNemar's tests, and biomarker correlations at the two time points were assessed using Spearman's rho and Pearson's correlation coefficients.

We used linear regression analysis with robust confidence intervals to determine if any of the biomarkers predicted (1) pre-ART CD4 count; (2) pre-ART VL; (3) post-ART CD4 count; or (4) CD4 count increase after ART initiation, adjusted for pre-ART CD4 count. Multivariate models included age and sex *a priori*. Age, CD4, and $\log_{10}$ -transformed VL were modeled as continuous variables. Models of post-ART outcomes also adjusted for time between pre- and post-ART samples, pre-ART CD4 count, and pre-ART VL. Additional variables were considered for multivariate models if the *p* value in bivariate analysis was < 0.20 . We planned *a priori* that if either HMGB1 or S100A9 was associated with an outcome, we would add LPS to the model to assess whether the effect of DAMPs on this outcome was mediated by LPS levels. Two-sided *p* values < 0.05 were considered significant.

¹Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, Washington.
Departments of ²Global Health and ³Epidemiology, University of Washington, Seattle, Washington.

Stata version 13.1 (StataCorp LP, College Station, Texas) was used for analysis.

Our study included 51 HIV-infected participants who initiated ART between 2003 and 2011. Study participants were 84% male, 73% men who have sex with men, and 35% injection drug users. At baseline, median age was 42 [IQR: 35, 49] years, pre-ART VL was 64,200 [IQR: 13,900, 154,000] copies/ml, and pre-ART CD4 count was 315 [IQR: 209, 378]. Median CD4 count increase after ART initiation was 326 [IQR: 117, 508] cells/ μ l, with a median of 1,105 (IQR: 894, 1,474) days between samples. Participants were on non-nucleoside reverse transcriptase inhibitor (NNRTI) ($n=23$), protease inhibitor (PI) ($n=20$), and integrase strand transfer inhibitor (INSTI)-based regimens ($n=8$) at post-ART sample. Eight (16%) participants changed regimens during the study period.

We detected a significant increase in mean HMGB1 levels from 1.95 ng/ml to 3.02 ng/ml after ART ($p=0.01$) (Supplemental Figure 1; Supplementary Data available online at www.liebertpub.com/aid). In stratified analysis, HMGB1 levels increased after ART in individuals taking PI and INSTI-based regimens, but did not change in those taking NNRTI-based regimens (data not shown). Because 80% of pre-ART S100A9 levels were BLQ of the assay (0.005 pg/ml), this variable was dichotomized. The proportion of individuals with detectable S100A9 increased significantly overall, from 19.6% before to 43.1% after ART ($p=0.01$) (Supplemental Figure 2). In stratified analysis, there were no significant differences in the proportion with detectable S100A9 within regimen classes (data not shown). There was no difference in mean LPS levels before and after viral suppression. We found no significant correlations between pre- and post-ART HMGB1 or S100A9 levels and no correlation between either HMGB1 or S100A9 and LPS levels at either time point (data not shown).

We found no significant associations between HMGB1, S100A9, or LPS and pre-ART CD4 count, adjusting for *a priori* confounders. Detectable post-ART S100A9 was independently associated with post-ART CD4 count in

adjusted models ($p=0.003$), whereas post-ART HMGB1 and post-ART LPS were not (data not shown).

Lastly, we sought to determine whether pre- or post-ART HMGB1, S100A9, or LPS or change in these biomarkers between time points was associated with CD4 count increase. Only detectable post-ART S100A9 met the criterion for multivariate analysis (Table 1). Detectable post-ART S100A9 remained significantly associated with a lesser CD4 count increase in multivariate modeling (Table 1). When pre and post-ART LPS were added individually to this model, neither was significant, and detectable post-ART S100A9 remained a significant predictor of immune reconstitution (data not shown).

To our knowledge, this is the first study to assess whether pre- or post-ART DAMP levels or changes in DAMP levels during viral suppression are associated with immune reconstitution. We found a significant association between detectable post-ART S100A9 and CD4 count increase and unexpectedly found that mean HMGB1 levels and proportion of individuals with detectable S100A9 increased after initiation of suppressive ART. Although HMGB1 levels increased in individuals taking PI-based or INSTI-based ART, there was no change in individuals on NNRTI-based regimens. These results suggest that mechanisms of inflammation in treated HIV infection may differ from those in untreated HIV infection and may also differ by ART regimen.

Our findings for HMGB1 are in contrast to a previous study that examined the effect of ART on HMGB1 and reported a 35% decrease in HMGB1 levels after virologic suppression.⁹ In that study, the effect was most apparent in participants with lower CD4 counts and higher VL at ART initiation. The discrepancy may be due to differences in patient populations or ART regimens used.

Because microbial translocation is likely involved in HIV pathogenesis and HMGB1 may facilitate LPS binding,¹⁰ we explored whether DAMP levels correlated with LPS levels, but found no correlation. In multivariate models, adjustment for LPS levels did not impact the association between detectable post-ART S100A9 levels and CD4 count increase,

TABLE 1. PREDICTORS OF CD4 RECONSTITUTION

Covariate	Bivariate model			Multivariate model		
	β coefficient	95% CI	p	β coefficient	95% CI	p
Male	-114.78	[-322.56, 93.00]	0.27	-8.19	[-217.71, 201.33]	0.94
Pre-ART age	-0.53	[-8.84, 7.78]	0.90	-1.05	[-9.25, 7.15]	0.80
Years between samples	82.26	[18.14, 146.37]	0.01	102.44	[34.38, 170.50]	0.004
Pre-ART CD4	-0.52	[-1.03, -0.005]	0.05	-0.34	[-0.82, 0.14]	0.16
Log ₁₀ Pre-ART HIV RNA	6.74	[-40.41, 53.88]	0.77	-37.25	[-149.52, 75.03]	0.51
Post-ART S100A9 detectable	-144.43	[-293.24, 4.38]	0.06	-222.58	[-364.55, -80.60]	0.003
HCV	-78.40	[-235.04, 78.23]	0.32			
HBV	36.06	[-357.95, 430.08]	0.86			
Pre-ART LPS	194.88	[-1110.13, 1499.89]	0.77			
Change in LPS	-33.98	[-1082.65, 1014.69]	0.95			
Post-ART LPS	294.93	[-1584.59, 2174.45]	0.75			
Pre-ART S100A9 detectable	5.90	[-186.79, 198.60]	0.95			
Change in S100A9	-0.85	[-7.93, 6.22]	0.81			
Pre-ART HMGB1	4.67	[-40.32, 49.66]	0.84			
Change in HMGB1	1.12	[-26.78, 29.02]	0.94			
Post-ART HMGB1	5.69	[-33.28, 44.66]	0.77			

ART, antiretroviral therapy; CI, confidence interval; LPS, lipopolysaccharide.

Bold highlighted results are statistically significant ($p < 0.05$).

suggesting that mechanisms other than LPS may drive inflammation in treated HIV infection.

Our study has a number of strengths. Although there was limited power to detect differences within ART classes, the study was adequately powered to detect overall differences in biomarker values. We used paired samples collected from patients who met strict inclusion criteria to minimize the possibility that other clinical conditions could confound our results. The clinical data were derived from a well-characterized cohort of HIV-infected individuals and all assays were performed in a laboratory by personnel with extensive experience performing similar analyses.

Our specimens were collected and processed according to AIDS Clinical Trials Group protocols. Although we cannot guarantee the timing of specimen storage after collection, we have no reason to believe it would vary between pre- and post-ART specimens. Our study population consists of mostly white men who have sex with men, who initiated ART with relatively preserved CD4 counts and maintained successful virologic suppression with ART. As such, our findings may not be applicable to other populations.

Overall, this study indicates that systemic DAMPs persist, and may increase following ART initiation in HIV-infected individuals. Moreover, elevated circulating DAMPs may contribute to sustained immune activation in ART-treated individuals, despite effective viral suppression. This DAMP immune activation may play a role in impaired immune reconstitution and may vary by ART regimen. Further investigation is needed to validate these results and define the role that DAMPs may play in the pathogenesis of HIV-associated conditions, such as cardiovascular disease and malignancies.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Daniel R. Drozd, MD, MSc
Division of Allergy and Infectious Diseases
Department of Medicine
University of Washington
Box 359931
Seattle, WA 98104

E-mail: ddrozd@uw.edu