LOW-DENSITY GRANULOCYTES DISPLAY IMMATURE CELLS WITH PRIMED PHENOTYPE IN PLWH

Juwon Park[1,2], Logan S. Dean[1,2], Jack Heckl[3], Michelle D. Tallquist[3], Dominic C. Chow[2,4], Cecilia M. Shikuma[1,2],
[1]Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burn School Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA
[2]Hawaii Center for AIDS, University of Hawaii at Manoa, Hawaii, USA, [3]Department of Cell and Molecular Biology, John A. Burn School Medicine, University of Hawaii at Manoa, Hawaii, USA,
[4]Department of Medicine, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA,

BACKGROUND

While a protective role of neutrophil extracellular traps (NET) in limiting HIV spread to susceptible cells has been documented, there is comparatively little insight into whether NET formation may also be harmful in people living with HIV (PLWH) as a potential proinflammatory driver of HIV-associated comorbidities. To gain insight into this issue, we assessed the frequency and characteristics of low-density granulocytes (LDGs), a subset of neutrophils and their NET forming capacity in PLWH on antiretroviral therapy (ART) and in HIV sero-negative (HIV-) individuals.

METHODS

Utilizing banked PBMCs from the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study, we identified and quantified circulating LDGs ($CD14^{-}CD16^{+}CD15^{+}$) by flow cytometry. We compared LDG maturation and activation, assessing CD10 expression and mean fluorescence intensity (MFI) of cell surface markers, CD11b, CD66b, and MPO per LDG, respectively. NET forming LDGs ($MPO^{+}H3Cit^{+}$) were analyzed and plasma levels of NET (H3Cit and cfDNA) were measured to detect NET components in blood. We also measured circulating inflammatory and prothrombotic markers to assess their relationship to plasma NET levels.

Table 1. Clinical and laboratory characteristics of people living with HIV and HIV-seronegative controls.

	HIV-seronegative (N=53)	HIV+ (N=88)	P-value
Demographics			
Age	55.0 [47.5-60.0]	49 [45-57]	0.03*
Male	46 (86.8%)	77 (87.5%)	0.90
Caucasian	34 (64.2%)	52 (59.1%)	0.55
Co-morbidities			
Body Mass Index (kg/m^2)	26.7 [22.9-29.8]	25.8 [23.9-27.9]	0.51
Past cigarette smoking	28 (54.9%)	54 (61.4%)	0.45
Current cigarette smoking	8 (15.1%)	16 (18.2%)	0.64
Diabetes	2 (3.8%)	8 (9.1%)	0.32
Hypertension	13 (24.5%)	30 (34.1%)	0.23
High cholesterol	19 (35.8%)	38 (43.2%)	0.39
History of myocardial infarction	1 (1.9%)	4 (4.5%)	0.65
History of stroke	0 (0%)	2 (2.3%)	0.53
Laboratory parameters and NET formation markers			
Hemoglobin (g/dL)	14.2 [13.6-15.0]	14.5 [13.5-15.45]	0.44
Platelet count (x10^9/L)	216 [180-256.5]	209 [175-233]	0.06
Total Leukocyte Count (x10^9/L)	5.20 [4.32-5.98]	5.1 [4.2-6.03]	0.59
Absolute Neutrophil Count (x10^3/uL)	3.02 [2.19-3.61]	2.74 [2.35-3.73]	0.82
CitH3 (ng/mL)	2.68 [1.96-5.28]	9.85 [4.64-13.0]	<0.001*
cfDNA (mg/mL)	0.71 [0.67-0.74]	0.68 [0.56-0.77]	0.06

Values presented are median [quartile 1, quartile 3] and n (%), as appropriate
*Statistically significant; P-values calculated using chi-square test or Fisher's Exact test for proportions and Mann-Whitney U test for medians

RESULTS

A total 141 subjects, (HIV+; n=88 and HIV-; n=53) were included in this study. The median (IQR) age was 49 (45.0-57.0) years (87.8% male) in HIV+ individuals and 55 (47.5-60.0) years (84.9% male) in the HIV- individuals. Most participant (89.0%) had undetectable HIV RNA (less than 50 copies/mL) and the median CD4+ T cell count was 469 (294.5-605.5) cells/mm^3. The number of LDGs was significantly decreased in HIV+ (*p=0.03*) compared to HIV- individuals (495.7 [262.5-1585], 1038 [442.3-3714], respectively). We observed significantly higher NET forming capacity in HIV+LDG compared to HIV- LDG. Moreover, HIV+ LDG displayed immature $CD10^{-}$ neutrophils, but had heightened expression of CD66b. Increased H3Cit levels (*p< 0.001*) were found in HIV+ individuals and these levels were positively associated with inflammatory and prothrombotic markers.

Figure 1

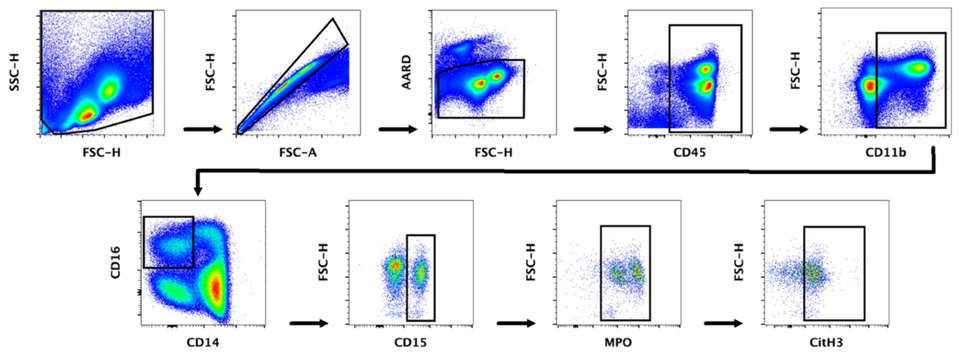


Figure 1. Representative gating strategy for identification of NET forming LDG populations from whole PBMC specimens.

Figure 2

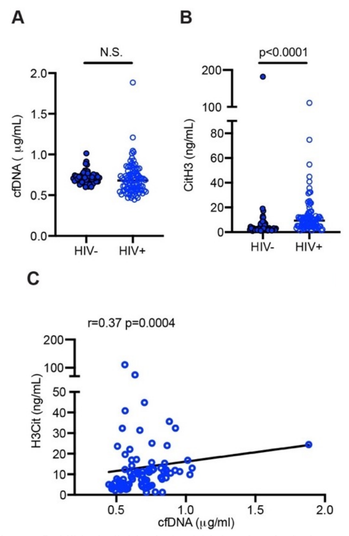


Figure 2. HIV+ individuals had higher level of plasma NETs compared to HIV- individuals. A-B. Plasma samples from HIV- individuals (n=58) and HIV+ individuals (n=88) were assessed for cfDNA (A) and CitH3 (B) C. Correlation between CitH3 and cfDNA in HIV+ individuals. (A, B) Mann-Whitney U test, N.S, indicates non-significant.

Table 2. Spearman's correlations between NET markers and hematological parameters and soluble biomarkers in HIV+ individuals.

		CitH3 (r value)	cfDNA (r value)
Hematological parameters	Total Leukocyte Count	0.095	0.298**
	Absolute Neutrophil Count	0.031	0.278**
	Platelet Count	0.129	0.061
Soluble biomarkers	CRP	0.234*	0.144
	MMP-9	-0.002	0.168
	E-Selectin	0.278**	0.089
	VCAM	0.155	0.089
	ICAM	0.062	-0.082
	Fibrinogen	0.251*	-0.027
	D-Dimer	0.115	0.067
	PAI	0.307**	0.269*
	MCP-1	0.260*	0.092
	TSP-1	-0.100	0.157
	MPO	0.237*	0.075
	TGF-β	-0.003	0.077
	SAA	0.275**	0.195
	SAP	0.169	0.131
	IL-1β	0.250*	0.198
	IL-6	0.322**	0.146
	IL-8	0.200	0.160
	IL-10	0.397**	0.168
	TNF-α	0.205	0.123

*p-value<0.05
**p-value<0.01

Figure 3

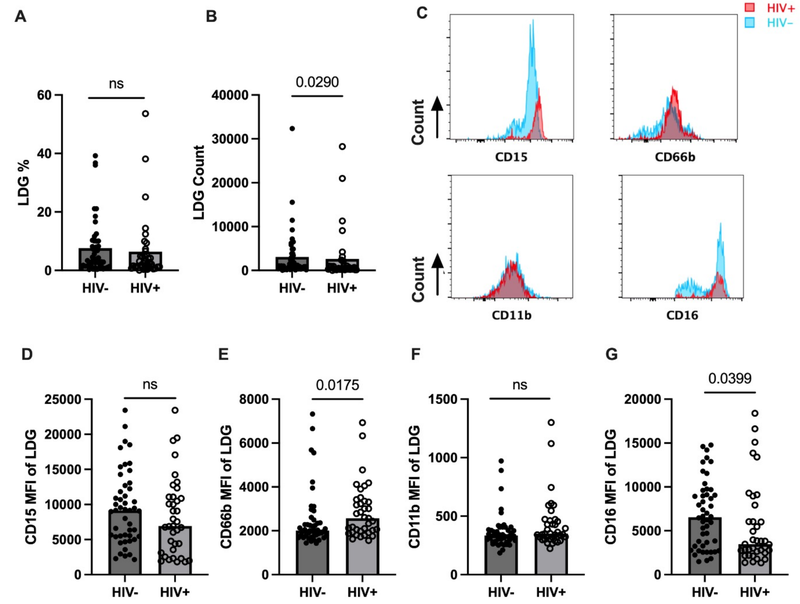


Figure 3. Characterization of LDG population in HIV+ and HIV- individuals **A-B**. Percentage (**A**) and numbers (**B**) of LDGs in HIV- and HIV+ individuals. **C.** Comparison of expression of neutrophil markers on LDGs between groups. Representative histograms of expression of CD15, CD66b, CD11b, and CD16 in LDGs in HIV- (blue) and HIV+ (red) groupsC). CD15 MFI (D), CD66b MFI (E), CD11b MIF (F), and CD16 MFI (G) of in HIV- and HIV+ individuals. Mann-Whitney U test; ns = p > 0.05.

Figure 4

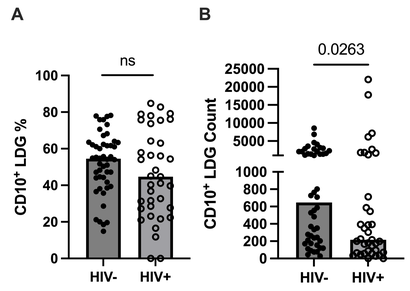


Figure 4. Decreased mature $CD10^{+}$ LDGs were observed in HIV+ individuals. The percentages **(A)** and counts of $CD10^{+}$ **(B)** in HIV- and HIV+ individuals. Mann-Whitney U test; ns = p > 0.05.

Figure 5

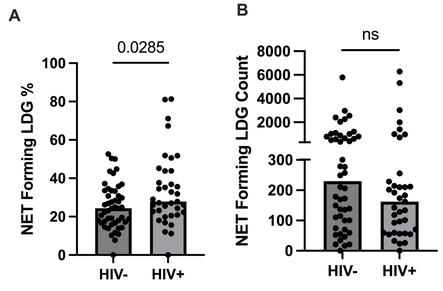


Figure 5. NET forming LDGs in HIV- and HIV+ individuals. **A-B**. NET forming LDG percentage (**A**), NET forming LDG count (**B**) in HIV- and HIV+ individuals. Mann-Whitney U test; ns = p > 0.05.

CONCLUSIONS

We found that LDGs from HIV+ individuals on ART display an immature and activated phenotype, and may exert proinflammatory effects. Plasma NET levels correlate with blood markers for inflammation and coagulation, which may yield insights into the pathologic role of LDGs at least in part mediated through NET formation in PLWH.