CD163⁺ Lymph Node Macrophages Are Correlated With Lymph Node Fibrosis in Chronic HIV

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Introduction

Despite being on effective antiretroviral therapy (ART), many HIV-infected individuals have impaired reconstitution of CD4 T cells. This is partly due to the fibrosis of lymphoid tissues (LT), which impedes signals important for T cell homeostasis. Macrophages are key regulators of tissue remodeling and fibrosis, though their involvement in LT fibrosis during chronic HIV has yet to be defined.

Study Design & Results

Inguinal lymph node (LN) excisions were performed by a board-certified surgeon on 6 virally-suppressed HIV-infected participants on ART and 4 HIV-uninfected participants recruited at the University Clinics at Kaka'ako at the John A. Burns School of Medicine. Immediately after excision, LN was divided into portions used for quantification of CD206⁺, CD163⁺, and CD169⁺ LN macrophage populations by multi-parametric flow cytometry and quantification of collagen fibers and CD4 T cells by IHC. Soluble markers of macrophage activation, fibrosis, and immune checkpoints were measured in cryopreserved plasma using ELISAs and Luminex.

Table 1. Baseline characteristics of study participants[a]

Demographic and Clinical Parameters	HIV+ (n=6)	HIV- (n=4)	*P*-value
Age, years	60 [53, 64]	51 [40, 53]	0.057
Male, n (%)	6 (100%)	4 (100%)	1.000
Caucasian, n (%)	3 (50%)	4 (100%)	0.076
HIV Viral load, n (%)			
Undetectable	2 (33%)	N/A	-
<20 HIV RNA copies	4 (67%)	N/A	-
Duration since HIV diagnosis, years	26 [18, 30]	N/A	-
Duration since ART initiation, years	19 [8, 25]	N/A	-
Immunologic Parameters Measured in Peripheral Blood			
Current CD4 T cell count, cells/μl	699 [402, 1068]	NC	-
Current CD4 T cell percent, %	35 [19, 49]	NC	-
Current CD8 T cell count, cells/μl	766 [667, 816]	NC	-
Current CD8 percent, %	37 [30, 44]	NC	-
CD4 CD8 T cell count ratio	0.9 [0.5, 1.6]	NC	-
Nadir CD4 T cell count, cells/μl	110 [7, 320]	N/A	-
sCD163, pg/mL	826 [579, 943]	681 [533, 820]	0.476
Neopterin, pg/mL	28 [20, 32]	21 [16, 26]	0.257
sCTLA-4, pg/mL	42 [37, 49]	33 [31, 40]	0.067
sPD-1, pg/mL	44 [35, 51]	39 [32, 41]	0.257
TGF-β_1, ng/mL	2.6 [2.4, 3.7]	1.4 [1.3, 1.6]	0.010*
TSP-1, ng/mL	219 [199, 278]	94 [42, 123]	0.010*
CICP, ng/mL	196 [172, 219]	93 [67, 143]	0.019*
Immunologic Parameters Measured in Lymph Node Tissue			
Collagen-1, % area in tissue	17 [13, 22]	20 [15, 39]	0.476
CD4 T cells, % area in tissue	32 [30, 39]	34 [19, 42]	0.914
CD169⁺ Macrophages, %	0.57 [0.26, 0.78]	0.31 [0.28, 0.34]	0.171
CD206⁺ Macrophages, %	0.27 [0.18, 0.38]	0.13 [0.11, 0.16]	0.100
CD163⁺ Macrophages, %	0.05 [0.01, 0.07]	0.02 [0.01, 0.03]	0.352

[a]Median values shown with [median Q1, median Q3] or frequency, n with (%); N/A, not applicable; NC, not collected

Figure 1. Flow cytometry gating strategy for identifying lymph node tissue macrophages (CD33⁺HLA-DR⁺) expressing CD169, CD163, or CD206

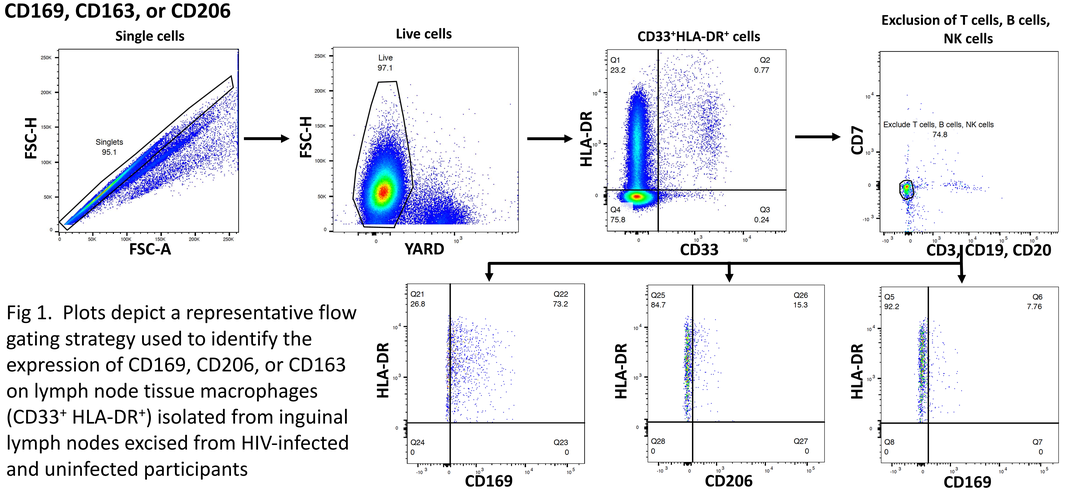


Fig 1. Plots depict a representative flow gating strategy used to identify the expression of CD169, CD206, or CD163 on lymph node tissue macrophages (CD33⁺ HLA-DR⁺) isolated from inguinal lymph nodes excised from HIV-infected and uninfected participants

Table 2. Pearson correlations between lymph node macrophage populations and other immunologic parameters measured in the peripheral blood and in lymph node tissue.

	CD169⁺ LN Macrophages	CD206⁺ LN Macrophages	CD163⁺ LN Macrophages
LN Collagen 1	r= 0.073 *p*= 0.841	r= 0.653 *p*= 0.041*	r= 0.694 *p*= 0.026*
LN CD4 T cells	r= -0.237 *p*= 0.509	r= -0.465 *p*= 0.176	r= -0.553 *p*= 0.097
Current CD4 T cell count	r= -0.624 *p*= 0.185	r= -0.566 *p*= 0.242	r= -0.827 *p*= 0.042*
CD4 CD8 T cell count ratio	r= -0.711 *p*= 0.113	r= -0.612 *p*= 0.196	r= -0.833 *p*= 0.039*
Nadir CD4 T cell count	r= -0.650 *p*= 0.235	r= -0.810 *p*= 0.097	r= -0.915 *p*= 0.030*
sCD163	r= 0.493 *p*= 0.148	r= 0.594 *p*= 0.070	r= 0.867 *p*= 0.001*
Neopterin	r= 0.747 *p*= 0.013*	r= 0.724 *p*= 0.018*	r= 0.736 *p*= 0.015*
sCTLA-4	r= 0.225 *p*= 0.532	r= 0.518 *p*= 0.125	r= 0.767 *p*= 0.010*
sPD-1	r= -0.074 *p*= 0.839	r= 0.325 *p*= 0.360	r= 0.713 *p*= 0.021*
TGF-β_1	r= 0.384 *p*= 0.273	r= 0.455 *p*= 0.187	r= 0.805 *p*= 0.005*
TSP-1	r= 0.444 *p*= 0.199	r= 0.553 *p*= 0.098	r= 0.740 *p*= 0.014*
CICP	r= 0.406 *p*= 0.244	r= 0.460 *p*= 0.181	r= 0.633 *p*= 0.049*

Summary

- HIV-infected participants were older than those uninfected, but not statistically significant. All participants were male and mostly Caucasian. All HIV-infected participants were virally suppressed with a median current CD4 count of 699 cells/ul, nadir CD4 count of 110 cells/ul, and CD4 CD8 T cell ratio of 0.9.
- CD163⁺ LN macrophages correlated with lower CD4 counts in blood and LN, as well as lower CD4 CD8 T cell ratios and nadir CD4 counts.
- CD163⁺ LN macrophages correlated with more collagen fibrils in LN and higher plasma levels of fibrosis biomarkers: TGF-b_1, TSP-1, CICP.
- CD163⁺ LN macrophages correlated with higher plasma levels of soluble immune checkpoints: CTLA-4 and sPD-1.
- CD163⁺ LN macrophages correlated with higher plasma markers of macrophage inflammation: sCD163 and Neopterin.

Conclusions

- CD163⁺ LN macrophages correlates with higher LN collagen deposition and lower CD4 T cell counts in the blood and LNs, which suggest that this macrophage subpopulation may be involved in the pathogenesis of LN fibrosis.
- CD163⁺ macrophages may be involved in both pro-inflammatory and pro-fibrotic responses, but additional studies are needed to investigate the dual role of this subpopulation in the context of LN fibrosis.

Methods

Immunohistochemistry
Immunohistochemistry of inguinal lymph nodes were performed by the Schacker laboratory as previously described (Sanchez et al, *JID* 2015). Briefly, Paraffin embedded LN tissues were sectioned and stained with Masson trichrome to identify collagen fibers, and CD4 antibody staining to identify CD4 T cells. Quantitative image analysis was used to measure the amount of tissue fibrosis and size of the CD4 T cell population.

Tissue macrophage phenotype staining assay
Freshly Isolated LN tissue cells were surface-stained with CD3, CD19, CD20, CD7, HLA-DR, CD33, CD206, CD169, and CD163 antibodies, and with Live/Dead fixable yellow dead cell stain (YARD). Data was acquired on a custom 4-laser BD LSRFortessa Cell Analyzer and all compensation and gating analyses were performed in FlowJo analytical software.

Detection of soluble plasma biomarkers by ELISA and Luminex
Whole blood was drawn into EDTA tubes and plasma was collected within one hour of collection on LN excision day and cryopreserved before use. Banked cryopreserved plasma aliquots were thawed and prepared according to kit manufacturer's instructions. sCD163, Neopterin, TSP-1 and CICP were measured by ELISA. sCTLA-4, sPD-1, and TGF-β_1 were measured by Luminex.

Statistical analysis
Man-Whitney tests, Chi-square tests, and Pearson's correlations were done in SPSS version 22 software and statistical significance was set at a *p*-value <0.05.

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