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Association of monocyte migration marker CD11b with pulmonary function in people living with HIV

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Abstract

Background: Maladaptive immune responses contribute to the pathogenesis of many chronic lung diseases. Here, we tested hypotheses that CD4 and CD8 T cell and monocyte phenotypes are associated with lung function in people living with HIV (PLWH) and those without HIV.

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Methods: Markers of T cell differentiation, activation, exhaustion and senescence, and markers of monocyte recruitment and migration were quantified in 142 HIV-positive and 73 HIV-negative participants of the Pittsburgh HIV Lung Cohort. All participants underwent lung function testing.

Results: CD4 or CD8 T cell phenotypes were not associated with measures of lung function in HIV-positive or HIV-negative participants after adjustment for multiple comparisons. In HIV-positive participants however, the percentage of classical monocytes that were $CD11b^+$ had positive associations at the Bonferroni-adjusted significance threshold of P=0.05/63 with pre- and post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio (β=0.36; P=0.00003 and β=0.31; P=0.0003, respectively). In stratified analyses of n=87 participants with CD4 500 cells/μL, associations of percentage of classical monocytes that were $CD11b^+$ with pre- and post-bronchodilator FEV_1/FVC ratio were stronger (β=0.48 and β=0.41, for pre- and post-, respectively) than in the entire HIV-positive study population. Significant associations of monocyte phenotypes were not observed in HIV-negative participants after adjustment for multiple comparisons.

Conclusions: $CD11b^+$ expression on classical monocytes is positively associated with FEV_1/FVC ratio in PLWH including in those with CD4 T cell recovery. Given the normal surveillance activity of monocytes, such association suggests this monocyte subset may play a role in preservation of pulmonary function in PLWH.

Keywords

T cell; monocyte; HIV; lung function; spirometry

INTRODUCTION

Impaired lung function and chronic lung diseases are common in people living with HIV (PLWH).[1] The incidence of non-communicable pulmonary disease, including chronic obstructive pulmonary disease (COPD) is also higher in PLWH compared to HIV-uninfected individuals in prospective studies, but the precise explanation for this observation is incompletely understood.[2–4] The extent to which antiretroviral therapy (ART) and CD4 T cell recovery impact lung function and mitigate lung disease risk is uncertain.

Mechanisms through which HIV infection contributes to non-infectious lung diseases are not fully understood. However, recent studies have considered whether chronic immune activation, a hallmark characteristic of HIV infection, may play an etiologic role.[5–11] ART allows patients to durably suppress HIV replication and achieve CD4 T cell recovery, but levels of immune activation biomarkers remain elevated in ART-treated and virally suppressed PLWH compared to HIV-uninfected individuals.[12,13] Residual immune activation in treated PLWH may contribute to several important comorbid conditions,[14] including pulmonary disease.

T cell surface markers (reflecting T cell activation and senescence) and plasma measures of systemic inflammation (C-reactive protein [CRP] and interleukin [IL]-6) have been associated with pulmonary dysfunction (lower pre- and post-bronchodilator forced expiratory volume in 1 second (FEV_1) and diffusing capacity of the lung for carbon

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monoxide [DL_{CO}]) in PLWH.[9] Plasma innate immune activation (soluble CD14 and soluble CD163) markers have been similarly associated with pulmonary dysfunction in PLWH.[5,6,8,10] Collectively, these data suggest both innate and adaptive arms of the immune response contribute to the pathogenesis of non-infectious lung diseases in PLWH.

In order to better understand associations of innate and adaptive immune markers with pulmonary function in PLWH, particularly in individuals with decades of durably suppressed HIV viremia and CD4 T cell recovery, we: (i) screened a large panel of T cell surface markers– reflecting T cell activation, differentiation, exhaustion and senescence; (ii) examined innate immune cell surface markers (classical, intermediate and non-classical monocytes and monocyte recruitment/migration markers) in HIV lung disease; and (iii) stratified specific analyses to measure potential heterogeneity of associations in individuals with and without CD4 T cell recovery. We tested the hypothesis that CD4 and CD8 T cell and monocyte cellular phenotypes are associated with lung function in PLWH, potentially with differential effects compared to similar people without HIV.

METHODS

Study participants

Participants from the Pittsburgh HIV Lung Cohort (previously reported in[15,16]) were included. Individuals in the Pittsburgh HIV Lung Cohort were recruited from the Pittsburgh AIDS Clinical Trial Unit, the Pittsburgh site of the Multicenter AIDS Cohort Study (MACS), or the San Francisco site of the Women's Interagency HIV Study (WIHS).[16,17] Exclusion criteria included pregnancy or breast-feeding; contraindication to pulmonary function testing; increasing respiratory symptoms or fevers (temperature>38°C) within 4 weeks of study entry; hospitalization within 4 weeks prior to study entry (excluding mental health); uncontrolled hypertension at screening visit (systolic >180 mmHg or diastolic >100 mmHg); current systemic chemotherapy or radiation for cancer; or current infection of the lungs, brain, or abdomen. All participants provided written, informed consent approved by institutional review boards at the sponsoring institutions.

Data collection

Demographic and clinical data were collected by trained interviewers and abstractors. These data included age, sex, race, ethnicity, self-reported cigarette smoking and duration, self-reported injection and non-injection drug use, history of bacterial or *Pneumocystis* pneumonia, and use of ART. Participants were considered 'ever-smokers' if they endorsed a history of smoking greater than 100 cigarettes in their lifetime. Participant CD4 T cell counts and HIV viral loads were determined via chart abstraction or from MACS or WIHS databases, if available, and the CD4 cell counts and viral loads obtained most proximal to pulmonary function testing (PFT) were recorded as current. Participants were categorized as having active hepatitis C virus (HCV) infection if they had both positive antibody testing and HCV viral RNA positivity as determined via review of laboratory testing. Blood samples were obtained at each study visit, processed and cryobanked until use.

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Pulmonary function testing

Participants performed pre- and post-bronchodilator spirometry and measurement of DL_{CO} per American Thoracic Society (ATS) and European Respiratory Society (ERS) standards.[18–20] DL_{CO}% was adjusted for hemoglobin and carboxyhemoglobin.[21] Percent-predicted lung function was determined using predicted values from the National Health and Nutrition Examination Survey (NHANES) III.[22] All calculations and interpretations were in accordance with the accepted reference standards of the ATS and ERS, and only tests meeting ATS standards or deemed acceptable by a trained pulmonologist as acceptable were included in the analysis.[18,21–24] Three participants had ATS-unacceptable spirometry and were excluded.

Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated immediately following blood collection using Histapaque-1077 (Sigma-Aldrich, St. Louis, MO) and were cryopreserved in liquid nitrogen in aliquots of 2 million cells per cubic centimeter of standard freezing medium (90% heat-inactive fetal bovine serum/10% dimethylsulfoxide). Cryopreserved PBMC were thawed, washed and immunostained for T cell and monocyte phenotyping. For lymphocyte subsets, the following antibody panel was used: LIVE/DEAD Aqua marker (Invitrogen, Carlsbad, CA), CD3-Alexa Fluor 700, CD4-BV421, CD8-CD650, CD45RA-APC, CCR7- PE-Cy7, HLADR-FITC, CD28-PE, CD38-PE-CF594, PD1-PerCP-Cy5.5, CD57-BV605, CD69-APC-Cy7 (all antibodies from BD Biosciences, Franklin Lakes, NJ). For monocyte subsets, the following antibody panel was used: LIVE/DEAD Aqua marker, CD14-APC-H7, CD16-FITC, CX3CR1-APC, HLADR-PE-Cy7, CD11b-PE, and an exclusion "dump" channel (combine CD3/CD19/CD56-Alexa 700) (all antibodies from BD Biosciences). All gates were defined with fluorescence-minus-one controls. Raw cytometry data were acquired using LSRFortessa X20 cytometer (BD Biosciences). Off-line analyses of cell populations were performed using FlowJo software (Treestar Inc, Ashland, OR). Procedures for cytometer calibration, on-line signal-to-noise compensation, live/dead cell discrimination, and off-line electronic gating of cells of interest including computation of fluorescence compensation matrices followed protocols that have been validated in a variety of clinical settings.[9,25–28]

Lymphocytes and monocytes gates were first determined using standard forward (FSC) and side scatter (SSC) profiles to ascertain cell size and granularity. The lymphocyte gate, containing CD4 and CD8 T cells, consisted of a cell cluster of lower FSC and SSC, whereas the monocyte gate was for the cluster with much larger magnitudes of FSC and SSC. After the exclusion of dead cells, $CD4^+$ and $CD8^+$ T cells were gated from $CD3^+$ cells. CD4 and CD8 T cell subsets were defined as: differentiation naïve ($CD45RA^+/CCR7^+$), central memory (CM) ($CD45RA^-/CCR7^+$), effector memory (EM) ($CD45RA^-/CCR7^-$) and terminally differentiated effector memory (TEMRA) ($CD45RA^+/CCR7^-$). Functional phenotypes were defined based on markers of general cellular activation ($CD38^+$; $HLA\text{-}DR^+$); exhaustion and exhaustion-associated inflammation ($PD1^+$; $CD69^+$); and senescence ($CD57^+$; $CD28^{null}$).

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A similar approach for the exclusion of dead cells and non-target cells (i.e. contaminating CD3^{-} CD4^{-} CD8^{-} CD16^{+} CD56$^{+/-}$ natural killer cells and CD19^{+} B cells) were used to examine monocyte subtypes in the gated larger FSC-SSC cluster. Monocyte subsets were defined as classical (CD14^{high}CD16^{-}), intermediate (CD14^{high}CD16^{+}), and non-classical (CD14^{+}CD16^{high}). Designation of recruitment and migration phenotypes of classical, intermediate, and non-classical monocyte subtypes were based on expression of CD11b and CX3CR1.

Statistical analysis

Bivariate associations of demographic, behavioral and clinical characteristics were assessed using t-tests (continuous variables) or chi-square or Fisher's exact tests (categorical variables) as appropriate. T cell and monocyte phenotypes were checked for normality and normalized with square root or natural log transforms as appropriate. Percent-predicted lung function measures were then analyzed for associations of T cell and monocyte phenotypes using linear regression models with adjustment for hepatitis B virus (HBV) infection (anti-HBc/HBsAg), HCV infection (anti-HCV+ and HCV RNA+), smoking pack-years, body-mass index (BMI), history of marijuana, crack cocaine use, injection drug use, and history of tuberculosis, history of bacterial pneumonia or *Pneumocystis* pneumonia (PCP). Prior studies of the relation of HBV, HCV and injection drug use with pulmonary function phenotypes have been limited and heterogeneous.[29–32] Associations of T cell and monocyte phenotypes with pulmonary function phenotypes were not qualitatively different with vs. without adjustment for HBV, HCV and injection drug use (data not shown).

Analyses of HIV-positive participants additionally included adjustment for current ART use, CD4 T cell count and HIV viremia status. We did not adjust our linear regression models for age because the percent-predicted pulmonary function outcomes were already age-adjusted. Bias associated with misspecified age in spirometry reference equations is well recognized[33] and in the current study we adjusted for exact age (pulmonary function date – date of birth / 365.25) rather than integer age. Standardized beta coefficients are presented for each linear regression analysis because the variability (e.g., interquartile range [IQR]) of T cell and monotype phenotypes was heterogeneous (Supplementary Tables 1–3).

We used Bonferroni thresholds to consider whether associations at the nominal level (P<0.05) might also be significant when adjusted for multiple comparisons. Specifically, we considered the number of hypothesis tests for CD4 T cells (16 cellular phenotypes * 7 pulmonary phenotypes), CD8 T cells (16 cellular phenotypes * 7 pulmonary phenotypes) and monocytes (9 cellular phenotypes * 7 pulmonary phenotypes) at Bonferroni significance thresholds of 0.05/112, 0.05/112 and 0.05/63, respectively, for HIV-positive and HIV-negative participants.

To measure potential heterogeneity of associations by immune status, we stratified analyses of HIV-positive participants by CD4 T cell count. We used two *a priori* defined thresholds for these analyses (i.e., <350 vs. 350 and <500 vs. 500 CD4 cells/μL). We did not consider heterogeneity of associations based on lower CD4 T cell count thresholds because few participants had low CD4 counts (i.e., n=10 [7%] of HIV-positive participants had CD4 T cell count <200 cells/μL).

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RESULTS

Study participant characteristics

One hundred and forty-two HIV-positive and 73 HIV-negative participants met the study inclusion criteria with available flow cytometry to determine T cell and monocyte phenotypes. HIV-positive and HIV-negative participants were similar with regard to most demographic and behavioral characteristics, aside from higher prevalence of injection drug and crack cocaine use and higher smoking pack-years in HIV-positive participants (Table 1). HIV-positive participants were also more likely to have a history of PCP treatment, lower post-bronchodilator FEV_1% and FVC% and lower DL_{CO}% than the HIV-negative participants (Table 1). Percentages of 8 of 16 CD4 T cell phenotypes, 10 of 16 CD8 T cell phenotypes and 2 of 9 monocyte phenotypes also differed between HIV-positive and HIV-negative participants (Supplementary Tables 1–3).

Associations of CD4 T cell phenotypes with lung function measures

In HIV-positive participants, three associations of CD4 T cell phenotypes with measures of lung function were observed at the nominal level (P<0.05). Specifically, percentage of $CD38^-$/$HLA\text{-}DR^-$ CD4 T cells was inversely associated with pre-bronchodilator FVC (β= –0.19; P= 0.03) whereas percentage of $CD38^+$/$HLA\text{-}DR^-$ CD4 T cells was positively associated with pre-bronchodilator FVC (β= 0.18; P= 0.042- Table 2). Percentage of $PD1^+$/$CD69^-$ CD4 T cells was also positively associated with post-bronchodilator FVC in HIV-positive participants (Table 2).

In HIV-negative participants, an inverse association at the nominal level of percentage $CD38^-$/$HLA\text{-}DR^-$ CD4 T cells with pre-bronchodilator FVC was also observed along with a positive association of percentage naïve and an inverse association of percentage CM CD4 T cells with pre-bronchodilator FVC (Supplementary Table 4).

Notably, none of the associations of CD4 T cell phenotypes in HIV-positive or HIV-negative participants were statistically significant after adjustment for multiple comparisons; Bonferroni threshold of P<0.05/112 hypothesis tests.

Associations of CD8 T cell phenotypes with lung function measures

In HIV-positive participants, percentage of CD8 T cells that were $CD38^-$/$HLA\text{-}DR^+$ was inversely associated at the nominal level (P<0.05) with six measures of lung function: pre- and post-bronchodilator FEV_1 (β= –0.30; P= 0.001 and β= –0.26; P= 0.004, respectively), pre- and post-bronchodilator FVC (β= –0.30; P= 0.001 and β= –0.21; P= 0.021, respectively) and pre- and post-bronchodilator FEV_1/FVC ratio (β= –0.18; P= 0.046 and β= –0.20; P= 0.029, respectively – Table 3).

In HIV-negative participants, two associations were observed at the nominal level of CD8 T cell phenotypes with measures of lung function. Specifically, percentage of CD8 T cells that were $CD38^+$/$HLA\text{-}DR^-$ was positively associated with post-bronchodilator FVC (β= 0.26; P= 0.038) and percentage of CD8 T cells that were $CD57^-$/$CD28^-$ was positively associated with pre-bronchodilator FEV_1 (β= 0.27; P= 0.029 – Supplementary Table 5).

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Similar to CD4 T cell analyses, none of the associations of CD8 T cell phenotypes in HIV-positive or HIV-negative participants were statistically significant after adjustment for multiple comparisons; Bonferroni threshold of P<0.05/112 hypothesis tests.

Associations of monocyte phenotypes with lung function measures

In HIV-positive participants, 12 of the 48 tested associations of monocyte phenotypes with measures of lung function were significant at the nominal level (Table 4). The most consistent associations of monocyte phenotypes in HIV-positive participants were with the percentage of classical monocytes that were CX3CR1^{+} (inverse associations with five of seven measures of lung function) and the percentage of classical monocytes that were CD11b^{+} (associations with four of seven measures of lung function – Table 4). Specifically, percentage of classical monocytes that were CX3CR1^{+} had inverse associations with pre- and post-bronchodilator FEV_1 (β= –0.28; P= 0.0011 and β= –0.22; P= 0.011), pre-bronchodilator FVC (β= –0.26; P= 0.002) and pre- and post-bronchodilator FEV_1/FVC ratio (β= –0.23; P= 0.008 and β= –0.21; P= 0.015). In contrast, while percentage of classical monocytes that were CD11b^{+} had positive associations with pre-bronchodilator FEV_1 (β= 0.30; P= 0.0009) and pre- and post-bronchodilator FEV_1/FVC ratio (β= 0.36; P=0.00003 and β= 0.31; P=0.0003), respectively), an inverse association with DL_{CO} (β= –0.21; P= 0.011) was observed (Table 4).

Percentage of classical monocytes that were CD11b^{+} had positive associations at the nominal level and at the Bonferroni-adjusted threshold of P=0.05/63 hypothesis tests with pre- and post-bronchodilator FEV_1/FVC ratio (Table 4). Significant associations of monocyte phenotypes were not observed in HIV-negative participants after adjustment for multiple comparisons (Supplementary Table 6).

Effect modification by immune status of monocyte phenotype associations

Associations of percentage of classical monocytes that were CD11b^{+} with pre- and post-bronchodilator FEV_1/FVC ratio in n=110 participants with CD4   350 cells/μL were similar if slightly stronger (β=0.43 and β=0.36, for pre- and post-, respectively - (Supplementary Table 7)) to associations observed in the entire HIV-positive study population (β=0.36 and β=0.31, for pre- and post-, respectively, Table 4). In n=87 participants with CD4   500 cells/μL, associations of percentage of classical monocytes that were CD11b^{+} with pre- and post-bronchodilator FEV_1/FVC ratio were stronger still (β=0.48 and β=0.41, for pre- and post-, respectively). In contrast, no significant associations of percentages of classical monocytes that were CD11b^{+} with FEV_1/FVC ratio were observed in participants with CD4 <350 cells/μL (n=24) or in participants with CD4 <500 cells/μL (n=47 – Supplementary Table 7).

DISCUSSION

In a detailed analyses of immune cell phenotypes in association with pulmonary function in persons with and without HIV infection, CD11b- and CX3CR1-expressing monocytes dominated. CD11b, one of four $β_2$ integrins, is a regulator of the trans-endothelial migration of blood monocytes into lymph nodes and other tissues. Its expression on

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monocytes has been considered an indicator of a pro-inflammatory phenotype,[34] implicating the role of $CD11b^+$ monocytes in HIV lung health. CX3CR1 is the receptor for the chemoattractive protein CX3CL1 or fractalkine. Its expression is therefore indicative of active migration. CX3CR1-CX3CL1 interaction is implicated in tissue injury/repair and in many inflammatory diseases.[35] In the setting of lung diseases such as COPD and in long-term smoking, $CX3CR1^+$ lung macrophages and monocytes have a role in disease pathophysiology.[36]

In the present work, associations of classical $CD11b^+$ monocytes with FEV_1/FVC ratio, a metric reflecting airflow, were statistically significant after adjustment for multiple comparisons. When low, FEV_1/FVC ratio may reflect persistent airflow limitation and chronic obstructive pulmonary disease (COPD).[37] Additionally, consistent associations at the nominal level ($P<0.05$) between measures of lung function and percentage of classical $CX3CR1^+$ monocytes, as well as percentage of $CD38^-$/HLA-DR^+ CD8 T cells, were observed in HIV-positive participants. Such associations were not observed in HIV-negative participants.

Data reported here supported our hypothesis that monocyte differentiation, recruitment and migration markers would be associated with measures of lung function in PLWH. Prior studies in the Pittsburgh HIV Lung Cohort and other populations observed that monocyte-derived receptors in plasma including soluble CD163 and soluble IL-2 receptor alpha (sIL-2Rα or CD25) are associated with the measures of pulmonary function assessed in the current investigation, including FVC and FEV_1/FVC ratio.[5,10] Additionally, soluble CD14, which may be a measure of microbial translocation or monocyte activation, was associated with pulmonary nodules and radiographic emphysema in prior studies.[6,8] The direction of the findings, however, was somewhat unexpected because CD11b expression on classically activated macrophages was associated positively with FEV_1/FVC ratio in the current investigation, suggesting association with better pulmonary function but inconsistent with prior data regarding “activated” monocytes and macrophages. This may be related to variability in the specific effects of CD11b-expressing cells.

CD11b plays a central role in the innate immune response including in anti-tumor immunity[38] and is the target of drug trials in pancreatic cancer.[39] In the setting of an experimental mouse model of acute lung injury, CD11b was inducibly expressed on alveolar macrophages that were recruited and derived from peripheral blood monocytes, and not on resident alveolar macrophages.[40] Following resolution of acute lung injury, a mixed pattern of CD11b and CD11c expression was observed on recruited alveolar lung macrophages[40] suggesting that these cells had transitioned to a more immunoregulatory phenotype. In murine models of a pediatric pulmonary disease mediated by inflammation following neonatal lung injury (bronchopulmonary dysplasia), CD11b expression on resident alveolar macrophages was protective against lung injury and loss of lung integrity,[41] consistent with an anti-inflammatory phenotype.

It is unclear why we observed positive associations of $CD11b^+$ classical monocytes with higher FEV_1/FVC ratio (suggestive of immunoregulatory function decreasing local inflammation), while finding an inverse association of the same monocyte subset at the

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nominal level with DL_{CO} (Table 4). Further investigation of monocyte and pulmonary resident macrophage polarization and phenotype may be revealing in regards to immune cell trafficking and dynamics of lung inflammation in PLWH.

CX3CR1 also plays a central role in recruitment of circulating monocytes into lung tissues. Pulmonary hypertension patients have higher CX3CR1 expression on blood monocytes compared to controls.[42] In a murine model, $CX3CR1^{+}$ mononuclear phagocytes initiate innate immune responses to cigarette smoke by producing TNF-α and IL-6.[43] However, lung $CX3CR1^{+}$ macrophages may also induce a suppressive phenotype and produce immunoregulatory cytokines such as IL-12 and IL-1.[36] One hypothesis is that $CD11b^{+}$/ $CX3CR1^{-}$ classical monocytes are associated with FEV1/FVC because they are specifically not recruited into the lung. However, associations of $CD11b^{+}$ on classical monocytes with FEV_1/FVC ratio were stronger than those of $CX3CR1^{+}$ and expression levels of these two molecules may reflect a single pathway as in this hypothesis or alternatively reflect distinct pathways that both affect FEV_1/FVC ratio.

The present data show associations of monocyte phenotypes varied by immune status. Specifically, the significant associations of $CD11b^{+}$ classical monocytes with measures of lung function were not observed in participants with low CD4 counts (i.e., CD4 <500 cells/μL (n=47, Supplementary Table 7). Indeed, in a stratified analyses of a subsample n=87 participants with CD4   500 cells/μL, associations of $CD11b^{+}$ classical monocytes with pre- and post-bronchodilator FEV_1/FVC ratio were stronger than in the entire HIV-positive study population. These data reinforce the complexity of HIV biomarker science, but they also suggest that continued evaluation and testing of monocyte migration markers in patients with pulmonary diseases and CD4 T cell recovery is valuable.

It was somewhat surprising to find that no significant T cell associations were observed following adjustment, given prior associations of activated $CD25^{+}$ CD4 and CD8 T cells with DL_{CO} and senescent $CD57^{+}$/$CD28^{null}$ CD8 T cells with post-bronchodilator FEV_1, amongst other observations.[9] We note, however, that the Pittsburgh HIV Lung Cohort has gotten older since we conducted that study (median age of HIV-positive participants of 46 years in the prior study vs. 53 years in the current study) and is less immune suppressed (median 535 vs. 596 CD4 T cells/μL in the current study). Another difference is inclusion of a slightly different panel of T cell markers that included immunostaining for CD38, HLA-DR, PD1 and CD69.

Particular strengths of our study include the careful phenotyping of innate and adaptive cell surface markers, including the first extensive characterization of monocyte markers in association with pulmonary function in PLWH, as well as a well-described cohort of participants with full spirometry and diffusing capacity. Our study also has several limitations. First, the sample size is relatively small, particularly when considering subclassifications of patients with low CD4 T cell counts. Additionally, this study is cross-sectional, which limits the interpretation of associations. There could be an interdependent relationship between immune activation and pulmonary disease, particularly since lung diseases including COPD predispose to regional dysbiosis that may underlie and drive local and systemic inflammation.[44] Finally, the nature of the study including extensive

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phenotyping of lymphocytes and monocytes, by necessity led to multiple comparisons. We recognize the limitations of Bonferroni and other commonly used correction methods, particularly because they assume that hypothesis tests are independent. Nonetheless, describing the data in their entirety including null and nominal associations will be useful to investigators with similar interests in immune cell phenotypes in pulmonary other organ dysfunction in the setting of HIV infection. Future studies will be needed to confirm our findings and to better describe the circumstances in which these surface markers and phenotypes develop and persist in PLWH with comorbidities.

In conclusion, this study suggests monocyte migration marker phenotyping including CD11b may be considered in the development of new biomarkers for pulmonary diseases in PLWH. If replicated in other populations and in mechanistic studies, these associations could serve to increase understanding of the pathogenesis of pulmonary diseases in PLWH, potentially leading to improved diagnoses and treatments in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic, behavioral and clinical characteristics of the study participants

	HIV-positive N=142	HIV-negative N=73	P value[a]
	n (%)	n (%)	
Female sex	31 (22)	22 (30)	0.18
Race/ethnicity			0.92
White, non-Hispanic	66 (46)	37 (51)	
Black, non-Hispanic	68 (48)	32 (44)	
Hispanic	5 (3)	3 (4)	
Other	3 (2)	1 (1)	
Ever smoker[b]	102 (72)	45 (62)	0.13
Illicit drug use, ever			
Marijuana	124 (87)	62 (85)	0.63
Crack cocaine	66 (47)	15 (21)	<0.001
Injection drug use	25 (18)	5 (7)	0.04
HBsAg+	16 (11)	5 (7)	0.30
HCV Ab+/RNA+	24 (17)	7 (10)	0.15
History of Tuberculosis	4 (3)	0	0.30
History of Pneumonia	33 (23)	18 (25)	0.82
History of PCP treatment	13 (9)	0	0.005
Current ART use	132 (93)	--	--
Undetectable HIV viral load	103 (73)	--	--
	Median (IQR)	Median (IQR)	
Age, year	53 (46–56)	51 (38–56)	0.08
Height, inches	69 (67–71)	69 (65–71)	0.15
BMI kg/m^2	25.7 (22.7–29.8)	26.8 (24.0–31.4)	0.21
Pack years[c]	12.0 (0–31.5)	3.2 (0–16.0)	0.007
CD4 count[d]	596 (418–867)	--	--
HIV viral load[e]	416 (116–4910)	--	--
Post-bronchodilator FEV1, liter	2.8 (2.3–3.5)	3.0 (2.4–3.5)	0.21
Post-bronchodilator FEV1%	87 (74–99)	93 (82–104)	0.02
Post-bronchodilator FVC, liter	3.6 (3.0–4.3)	3.8 (3.1–4.3)	0.17
Post-bronchodilator FVC%	86 (76–96)	90 (84–101)	0.002
Post-bronchodilator FEV1/FVC%	81 (76–84)	81 (78–84)	0.82
DL_{CO} %	74 (66–82)	84 (76–93)	<0.001

HBsAg: hepatitis B virus surface antigen; HCV Ab: hepatitis C virus antibody; PCP: Pneumocystis carinii pneumonia; BMI: body-mass index; ART: antiretroviral therapy; FEV1: forced expiratory volume in 1 second; FVC: forced expiratory capacity; DL_{CO}; diffusing capacity for carbon monoxide

[a]Using t-tests (for continuous variables) or Chi-square or Fisher's exact tests as appropriate (categorical variables)

[b] Ever smokers reported lifetime history of smoking >100 cigarettes

[c] Amongst ever smokers

[d] Among n=134 participants with available CD4 T cell data

[e] In 31 patients with detectable VL.

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Table 2.

Associations of CD4 T cell phenotypes with pulmonary function measures, HIV-positive participants (n=133 with pre-; n=131 with post-)[a, b, c, d]

		FEV1		FVC		FEV1/FVC		DL_{CO}
		Pre-FEV1%	**Post-FEV1%**	**Pre-FVC%**	**Post-FVC%**	**Pre-FEV1/FVC**	**Post-FEV1/FVC**	**DL_{CO}%**
Differentiation	% Naïve	0.08	0.06	0.11	0.08	0.03	0.01	−0.03
	% CM	−0.04	−0.03	−0.07	−0.08	0.06	0.08	0.08
	% EM	−0.01	0.02	−0.07	−0.02	0.01	0.05	−0.01
	% TEMRA	−0.07	−0.06	−0.08	−0.04	−0.02	−0.03	−0.04
Activation Profiles	% CD38− & HLA-DR−	−0.15	−0.12	**−0.19** *	−0.15	−0.06	−0.05	−0.03
	% CD38+ & HLA-DR−	0.15	0.13	**0.18** *	0.14	0.07	0.05	0.04
	% CD38− & HLA-DR+	−0.10	−0.09	−0.13	−0.09	0.002	−0.001	−0.12
	% CD38+ & HLA-DR+	−0.01	0.002	−0.02	−0.01	0.03	0.05	−0.05
Inflammation/Exhaustion Profiles	% PD1− & CD69−	−0.13	−0.14	−0.15	−0.15	−0.08	−0.10	−0.05
	% PD1+ & CD69−	0.14	0.16	0.16	**0.17** *	0.10	0.11	0.03
	% PD1− & CD69+	0.03	0.02	0.08	0.03	−0.01	0.01	0.01
	% PD1+ & CD69+	0.11	0.11	0.13	0.11	0.08	0.11	−0.03
Senescence Profiles	% CD57− & CD28−	−0.08	−0.07	−0.10	−0.06	−0.06	−0.08	−0.05
	% CD57+ & CD28−	−0.07	−0.06	−0.09	−0.05	−0.04	−0.05	0.03
	% CD57− & CD28+	0.08	0.06	0.09	0.02	0.05	0.08	−0.03
	% CD57+ & CD28+	−0.05	−0.03	−0.11	−0.06	−0.01	−0.02	0.00

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; DL_{CO}; diffusing capacity for carbon monoxide; CM: central memory; EM: effector memory; TEMRA: terminally differentiated effector memory

[a]Percent-predicted lung function was determined using predicted values from NHANES III.[22]

[b]CD4 T cell phenotypes were checked for normality and normalized with square root or natural log transforms as appropriate

[c]Percent-predicted lung function measures were analyzed for associations with T cell phenotypes using linear regression models with adjustment for HBV infection (anti-HBc/HBsAg), HCV infection (anti-HCV+ & HCV RNA+), smoking pack-years, body-mass index (BMI), history of marijuana, crack cocaine and injection drug use and history of tuberculosis, pneumonia or treatment for pneumocystis carinii pneumonia (PCP), current ART use, CD4 T cell count and HIV viremia status.

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[d] Standardized beta coefficients are presented for each linear regression analysis because the variability (e.g., interquartile range (IQR)) of CD4 T cell phenotypes was heterogeneous (Supplementary Table 1).

[*] = 0.01 $P < 0.05$

[§] = 0.001 $P < 0.01$

[†] = $P < 0.001$

[‡] $P <$ Bonferroni significance threshold of P=0.05/112 hypothesis tests

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Table 3.

Associations of CD8 T cell phenotypes with pulmonary function measures, HIV-positive participants (n=133 with pre-; n=131 with post-)[a, b, c, d]

		FEV1		FVC		FEV1/FVC		DL_{CO}
		Pre-FEV1%	**Post-FEV1%**	**Pre-FVC%**	**Post-FVC%**	**Pre-FEV1/FVC**	**Post-FEV1/FVC**	**DL_{CO}%**
Differentiation	% Naïve	–0.02	–0.06	0.02	–0.03	0.03	0.008	–0.03
	% CM	0.06	0.05	0.03	–0.005	0.04	0.07	–0.06
	% EM	0.14	0.16	0.06	0.07	0.13	0.15	0.03
	% TEMRA	–0.10	–0.06	–0.04	0.02	–0.12	–0.11	–0.01
Activation Profiles	% CD38– & HLA-DR–	–0.07	–0.04	–0.13	–0.10	–0.03	0.008	0.01
	% CD38+ & HLA-DR–	–0.10	0.04	0.14	0.06	0.05	0.03	–0.03
	% CD38– & HLA-DR+	**–0.30** §	**–0.26**§	**–0.30** §	**–0.21** *	**–0.18** *	**–0.20** *	–0.11
	% CD38+ & HLA-DR+	–0.14	–0.11	–0.09	–0.02	–0.12	–0.14	–0.09
Exhaustion Profiles	% PD1– & CD69–	–0.05	–0.09	–0.08	–0.14	0.008	0.005	–0.01
	% PD1+ & CD69–	0.07	0.12	0.10	0.17	–0.01	–0.02	0.01
	% PD1– & CD69+	–0.11	–0.12	–0.06	–0.04	–0.12	–0.15	–0.09
	% PD1+ & CD69+	–0.02	–0.004	0.02	0.05	–0.07	–0.08	–0.11
Senescence Profiles	% CD57– & CD28–	–0.06	–0.06	–0.01	0.01	–0.07	–0.12	–0.07
	% CD57+ & CD28–	0.11	0.12	0.10	0.12	0.02	0.03	0.001
	% CD57– & CD28+	–0.07	–0.08	–0.05	–0.09	–0.02	–0.009	0.02
	% CD57+ & CD28+	–0.05	0.03	–0.11	0.001	0.04	0.04	0.06

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; DL_{CO}: diffusing capacity for carbon monoxide; CM: central memory; EM: effector memory; TEMRA: terminally differentiated effector memory

[a]Percent-predicted lung function was determined using predicted values from NHANES III.[22]

[b]CD8 T cell phenotypes were checked for normality and normalized with square root or natural log transforms as appropriate

[c]Percent-predicted lung function measures were analyzed for associations with T cell phenotypes using linear regression models with adjustment for HBV infection (anti-HBc/HBsAg), HCV infection (anti-HCV+ & HCV RNA+), smoking pack-years, body-mass index (BMI), history of marijuana, crack cocaine and injection drug use and history of tuberculosis, pneumonia or treatment for pneumocystis carinii pneumonia (PCP), current ART use, CD4 T cell count and HIV viremia status.

[d] Standardized beta coefficients are presented for each linear regression analysis because the variability (e.g., interquartile range (IQR)) of CD4 T cell phenotypes was heterogeneous (Supplementary Tables 1).

[*] = 0.01 $P < 0.05$

[§] = 0.001 $P < 0.01$

[†] = $P < 0.001$

[‡] $P <$ Bonferroni significance threshold of P=0.05/112 hypothesis tests

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Table 4.

Associations of monocyte subsets with pulmonary function measures, HIV-positive participants (n=144 with pre-; n=142 with post-)[a, b, c, d]

			FEV1		FVC		FEV1/FVC		DL_{CO}
			Pre-FEV1%	**Post-FEV1%**	**Pre-FVC%**	**Post-FVC%**	**Pre-FEV1/FVC**	**Post-FEV1/FVC**	**DL_{CO}**
	Monocyte subsets	% Classical	0.02	0.10	0.06	0.12	−0.06	−0.02	0.08
		% Intermediate	−0.03	−0.08	−0.05	−0.10	0.09	0.06	**−0.18** *
		% Non-Classical	−0.008	0.04	−0.08	−0.10	0.13	0.12	−0.13
Cell recruitment & migration markers	Classical	%CD11b+	**0.30** §	0.13	0.17	−0.09	**0.36** †,‡	**0.31** †,‡	**−0.21** *
		%CX3CR1+	**−0.28** §	**−0.22** *	**−0.26** §	−0.15	**−0.23**§	**−0.21** *	−0.007
	Intermediate	%CD11b+	−0.15	−0.15	−0.15	−0.10	−0.16	**−0.19** *	−0.15
		%CX3CR1+	0.02	0.004	0.02	0.001	0.02	0.008	−0.004
	Non-classical	%CD11b+	0.05	0.06	0.10	0.10	−0.05	−0.04	0.10
		%CX3CR1+	−0.11	−0.10	−0.10	−0.06	−0.16	**−0.19** *	−0.13

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; DL_{CO}; diffusing capacity for carbon monoxide

[a]Percent-predicted lung function was determined using predicted values from NHANES III.[22]

[b]Monocyte phenotypes were checked for normality and normalized with square root or natural log transforms as appropriate

[c]Percent-predicted lung function measures were analyzed for associations with monocyte phenotypes using linear regression models with adjustment for HBV infection (anti-HBc/HBsAg), HCV infection (anti-HCV+ & HCV RNA+), smoking pack-years, body-mass index (BMI), history of marijuana, crack cocaine and injection drug use and history of tuberculosis, pneumonia or treatment for pneumocystis carinii pneumonia (PCP), current ART use, CD4 T cell count and HIV viremia status.

[d]Standardized beta coefficients are presented for each linear regression analysis because the variability (e.g., interquartile range (IQR)) of CD4 T cell phenotypes was heterogeneous (Supplementary Table 3).

* = 0.01 $P < 0.05$

§ = 0.001 $P < 0.01$

† = $P < 0.001$

‡ $P <$ Bonferroni significance threshold of P=0.05/63 hypothesis tests