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Relationships of pulmonary function, inflammation, and T-cell activation and senescence in an HIV-infected cohort

Meghan E. FITZPATRICK¹, Vikas SINGH¹, Marnie BERTOLET³, Lorrie LUCHT¹, Cathy KESSINGER¹, Joshua MICHEL⁴, Alison LOGAR⁴, D. Renee WEINMAN¹, Deborah MCMAHON¹, Karen A. NORRIS², Abbe N. VALLEJO^{2,4}, and Alison MORRIS^{1,2}

¹Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

²Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA

³University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA

⁴Department of Pediatrics, University of Pittsburgh, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA

Abstract

Objective—To determine associations between circulating markers of immune activation, immune cell senescence, and inflammation with HIV-associated abnormalities of pulmonary function.

Design—HIV infection is an independent risk factor for abnormal pulmonary function. Immune activation, immune senescence, and chronic inflammation are characteristics of chronic HIV infection that have been associated with other HIV-associated comorbidities and may be related to pulmonary disease in this population.

Methods—Participants from an HIV-infected cohort (n=147) completed pulmonary function testing (PFT). Markers of T-cell activation and senescence were determined by flow cytometry,

Corresponding Author: Meghan Fitzpatrick, Department of Medicine, 3459 Fifth Avenue, NW 628 MUH, Pittsburgh, PA 15213, fitzpatrickme2@upmc.edu.

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LL: acquisition of data; revising for intellectual content, final approval for publication, agreement to accountability of work

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and plasma levels of interleukin (IL)-6, IL-8, and C-reactive protein (CRP) were measured, as was telomere length of peripheral blood mononuclear cells (PBMC). Regression models adjusting for clinical risk factors were constructed to examine relationships between biomarkers and PFT outcomes.

Results—Activated CD25⁺ T-cells and activated/senescent CD69⁺/CD57⁺/CD28^{null} CD4⁺ T-cells, IL-6, and CRP were associated with PFT abnormalities. Shortening of PBMC telomere length correlated with airflow obstruction and diffusing impairment. Paradoxically, circulating senescent CD57⁺/CD28^{null} CD8⁺ T-cells were associated with better PFT outcomes.

Conclusions—Circulating T-cells expressing markers of activation and inflammatory cytokine levels are independently correlated with PFT abnormalities in HIV-infected persons. Overall telomere shortening was also associated with pulmonary dysfunction. The paradoxical association of senescent CD8⁺ T-cells and better PFT outcomes could suggest an unrecognized beneficial compensatory function of such cells or a redistribution of these cells from the circulation to local compartments. Further studies are needed to differentiate and characterize functional subsets of local pulmonary and circulating T-cell populations in HIV-associated pulmonary dysfunction.

Keywords

HIV; Pulmonary Disease; Chronic Obstructive; Aging; Inflammation; T-lymphocytes

INTRODUCTION

For HIV-infected individuals with access to treatment, HIV has become a chronic disease[1], and older HIV-infected persons face a host of comorbidities, including age-related diseases that may be accelerated by premature cellular senescence, immune activation, and inflammation associated with HIV[2–4]. Chronic obstructive pulmonary disease (COPD) is one of these important comorbidities. Although epidemiologic data have established a link between HIV and COPD[5–13], the mechanism(s) by which COPD develops in HIV-infected persons is unknown. HIV and COPD, independent of each other, are known to exhibit varying states of immune activation, cellular senescence, and systemic inflammation; conditions that could amplify disease manifestations within the setting of HIV-associated COPD.

COPD is common in the HIV-infected population. Epidemiologic studies have reported prevalence of physician diagnosis of COPD, abnormal spirometry, or abnormal diffusing capacity ranging from 17–60% of HIV-infected persons[6,7,11,12,14]. In addition to being prevalent, COPD is likely also accelerated in HIV. Recent studies indicate the mean age of those with COPD was 49 years[15], and severe emphysema in HIV-infected persons has been reported in the fourth decade of life[8]. Effects of antiretroviral therapy (ART) on lung function are unclear, but the pattern of prevalent and early COPD has persisted in the current era[10,15]. Although cigarette smoking is very common among persons with HIV, the attributable risk secondary to HIV is independent of smoking or other risk factors, including injection drug use or prior pulmonary infection[6,15].

Despite the importance of COPD in HIV, little is known regarding the mechanisms of its development. COPD in HIV infection may be in part driven by accelerated immune activation and/or senescence in HIV-infected persons. T-cells in HIV-infected individuals have features of cell senescence and chronic activation[16–18]. Senescent T-cells have maladaptive function and may contribute to upregulation of the inflammatory response independent of triggering of the T-cell receptor [19,20]. Premature senescence of lymphoid and non-lymphoid cells occurs in HIV-infected individuals and has been linked to a number of comorbid conditions such as cardiovascular disease, neurocognitive disorders, osteoporosis/osteopenia, and renal insufficiency[21–24], conditions that are typically seen in older HIV-uninfected adults[25]. COPD may be related to immune senescence in HIV given both its age-related prevalence and its pathogenic link to aging in the HIV-uninfected population[26–31]. Abnormal T-cell activation, detected via surface expression of immune activation markers, is a feature of HIV infection that is not completely ameliorated by ART, even with effective viral suppression[32]. Persistent immune activation has been associated with HIV progression and worse survival[33] as well as with presence of cardiovascular comorbidity among HIV-infected persons[34–36]. Additionally, circulating biomarkers associated with a persistent inflammatory phenotype are elevated among persons with HIV[37]. Inflammatory biomarkers in HIV-infected persons (including C-reactive protein[CRP] and interleukin[IL]-6) have been associated with worse mortality outcomes[38] and may mediate comorbidity-specific outcomes such as cardiovascular disease[39]. In the HIV-uninfected population, relationships between COPD and inflammatory mediators have been established[40,41], though the drivers of systemic inflammation are not readily apparent.

In this study, we investigate relationships among markers of T-cell activation (CD38⁺, CD25⁺, CD69⁺), immune senescence (CD57⁺/CD28^{null}, telomere shortening), and circulating markers of inflammation (IL-6, IL-8, CRP) with pulmonary function abnormalities typical of COPD in an HIV-infected cohort.

METHODS

Subjects

Participants were recruited from an existing cohort that enrolled HIV-infected individuals 18 years of age and older from the University of Pittsburgh/Pittsburgh AIDS Center for Treatment Clinic[11]. Participants were screened and excluded if there were contraindications to undergoing pulmonary function testing (PFT), if they had new or increasing respiratory symptoms, or if they had fever within 4 weeks prior to enrollment. Participants signed written informed consent, and study protocols were approved by the Institutional Review Board of the University of Pittsburgh. Participants were enrolled in the current study between July 2007 and June 2011, and followed prospectively through 36 months, with data collection taking place at baseline, 18-month, and 36-month time points. The current cross-sectional analysis uses data generated from the 18-month study visit, which was the first visit with an adequate number of peripheral blood mononuclear cell (PBMC) samples.

Data collection

Demographic and clinical data were collected by trained interviewers. These data included age, gender, race, ethnicity, self-reported injection drug use, self-reported cigarette smoking and duration, history of bacterial or *Pneumocystis* pneumonia, and use of and adherence to antiretroviral drugs. Participants were considered “ever-smokers” if they endorsed a history of smoking greater than 100 cigarettes. Participant CD4⁺ T-cell counts and HIV viral loads were determined via chart abstraction, and the CD4⁺ counts and viral loads obtained most proximal to PFT were recorded as current. Participants were categorized as having active hepatitis C if they had both positive antibody testing and viral RNA positivity as determined via review of laboratory testing. Blood samples were obtained using standard procedures at each study visit and were processed and banked for future testing.

Pulmonary function testing

Spirometry was performed before and after administration of 400µg of albuterol by inhalation (4 puffs from a metered dose inhaler) following American Thoracic Society (ATS)/European Respiratory Society (ERS) standards[42]. Participants also performed a single breath determination of carbon monoxide uptake in the lungs to measure diffusing capacity for carbon monoxide (DL_{CO}) per ATS/ERS standards[43]. Spirometry reference values were determined from the third National Health and Nutrition Examination Survey (NHANES III) equations[44], and reference values for DL_{CO} used Neas *et al.* equations[45] that were adjusted for hemoglobin and carboxyhemoglobin measured from blood testing at the time of the pulmonary function testing[43].

Flow cytometry

T-cell surface markers were analyzed from frozen PBMC stored in liquid nitrogen. Viability was determined via dye exclusion of trypan blue, and samples were rejected if viability was <30%. Approximately 500,000 cells per sample were analyzed for the presence of cell surface markers (allophycocyanin anti-TCRα/β [Biolegend, San Diego, CA], brilliant violet 421 anti-CD4 [BD Biosciences, San Jose, CA], Qdot® 605 anti-CD8 [Life Technologies, Grand Island, NY]), activation markers (peridinin chlorophyll protein-cyanin 5.5 anti-CD25 [BD Biosciences], phycoerythrin-cyanin 7 anti-CD38 [BD Biosciences], allophycocyanin – cyanin 7 anti-CD69 [BD Biosciences]), and senescence markers (fluorescein isothiocyanate anti-CD57 [BD Biosciences] and phycoerythrin anti-CD28 [BD Biosciences]). Multicolor flow cytometry was performed according to previously established protocols[19,20] using a FACSaria II cytometer (Becton Dickinson, San Francisco, CA), and the collected cytometry data were analyzed offline with FlowJo 7.6.5 (Tree Star, Inc., Ashland, OR). Following matrix compensation to fluorochrome-stained 3.27 µm control beads (Spherotech, Lake Forest, IL), the live lymphocyte population was gated from forward and side scatter profiles. Background staining was minimized, and detection signals were optimized by the inclusion of isotype antibody controls, and unstained/single stain cell controls in each cytometry experiment. T-cells were defined as TCRα/β⁺, CD4⁺ or CD8⁺. To minimize variance, subsets of populations containing less than 100 cells were not included in the analysis. Senescent T-cells were defined as those with the expression pattern CD57⁺/CD28^{null}.

Activation markers were assessed both independently and co-expressed with senescence markers.

Circulating biomarker measurements

Plasma levels of CRP and cytokines IL-6 and IL-8 were assayed with commercially available high-sensitivity ELISA kits (CRP: Phoenix Pharmaceuticals, Burlingame, CA; IL-6 and IL-8: R&D Systems, Minneapolis, MN) following the manufacturers' protocols. All ELISA were performed in triplicate.

Telomere length determination

DNA was isolated and purified from PBMCs stored at -80°C using the QIAgen DNEasy Blood and Tissue Kit (QIAgen, Valencia, CA), per manufacturer's instructions. DNA was quantified and assessed for purity using spectrophotometry. Relative telomere length was determined as a T/S ratio using quantitative real time polymerase chain reaction (qPCR) as described by Cawthon[46], with minor modifications to the cycle length to optimize for a BioRad PCR machine. All qPCR reactions were run in concert with a standard curve and a negative control. One sample of DNA was selected arbitrarily from the cohort to serve as the control for relative ratio calculations. Samples were performed in triplicate using 60ng of template DNA. Triplicate results were assessed for coefficient of variation (CV). Results showing a CV of $\geq 20\%$ were discarded, and qPCR was repeated.

Statistical analysis

Statistical analyses were performed using Stata 12 (StataCorps, College Station, TX). Cytometry data were expressed as a percentage of the parent population. Biomarker levels were analyzed as continuous variables if normally distributed and were otherwise dichotomized. CRP was dichotomized at 1 mg/L, and other variables were split at the 75th percentile with values less than the 75th percentile identified as the reference. To assess relationships between flow cytometry marker percentages, circulating biomarker levels, and PFTs, we created multivariable models for each biologic variable with each of the following PFT outcomes: percent-predicted FEV₁ (pre- and post-bronchodilator), FEV₁/FVC ratio (pre- and post-bronchodilator), and percent-predicted DL_{CO}. PFT variables were also dichotomized above and below clinically relevant cutpoints for airflow obstruction (FEV₁/FVC above and below 0.7) and DL_{CO} (percent-predicted DL_{CO} above and below 0.6; moderate impairment). Potentially relevant confounding variables included age, race, ethnicity, gender, pack-years smoked, duration of HIV, use of anti-retroviral therapy, viral load, CD4 cell count, bacterial or *Pneumocystis* pneumonia history, injection drug use history, and BMI. Appropriately transformed variables were selected using a forward stepwise approach with p-to-enter = 0.05 and p-to-exit = 0.10. Age, race, ethnicity, and gender were not used for models with percent-predicted PFT outcomes, as percent-predicted values already include adjustment for these variables. For examination of associations with lower numbers of observations, confounders were omitted from final models if necessary to maintain stability of the model. Pearson or Spearman correlations were used to examine associations between selected biomarkers demonstrating relationship to pulmonary function

in the multivariable models. Variables were log-transformed as necessary to approximate normality.

RESULTS

Characteristics of the cohort

147 HIV-infected persons completed acceptable pulmonary function testing (PFT) and were included in the analysis. Mean age of participants was 45.5 years, the majority (66.7%) were male, and most (82.9%) were either current or former smokers, with a median of 10 pack years smoked (Table 1).

Spirometry values in the cohort were normal on average (Table 1); however, 28.8% of participants had evidence of airflow obstruction (FEV_1/FVC below 0.7). DL_{CO} was low on average (mean percent-predicted DL_{CO} 0.67), and there was a high prevalence of diffusing impairment: 86.4% of participants had $DL_{CO} < 80\%$ predicted (reduced) and 34.0% had $DL_{CO} < 60\%$ predicted (moderately reduced).

Immune activation, inflammation, and senescence in relationship to pulmonary function

All study participants did not have every biomarker due to limited sample availability, and individual analyses of relationships between biomarkers and PFT outcomes were restricted to those with available data. Because cytometry data were not included in the analysis if the parent population consisted < 100 cells, there were frequently fewer data available for the smaller $CD4^+$ populations as might be expected in HIV. Median values and interquartile ranges (IQR) are presented in Table 2.

DL_{CO}

Elevated ($> 75^{th}$ percentile) $CD25^+/CD4^+$, $CD25^+/CD8^+$, and $CD69^+/CD57^+/CD28^{null} CD4^+$ T-cell percentages were associated with lower DL_{CO} as a continuous measure, as were elevated plasma IL-6 levels and levels of CRP > 1 mg/L (Table 3). When DL_{CO} was analyzed as a dichotomous variable, elevated $CD25^+/CD4^+$ and $CD25^+/CD8^+$ percentages were associated with greater odds of moderately impaired DL_{CO} . In contrast, and unexpectedly, higher $CD57^+/CD28^{null} CD8^+$ T-cell percentages were associated with lower odds of moderate diffusing impairment. Of the inflammatory markers, IL-6 and CRP > 1 mg/L were associated with greater odds of moderately impaired DL_{CO} (Table 4).

Spirometry

Lower pre-BD FEV_1 percent-predicted (continuous) was associated with elevated ($> 75^{th}$ percentile) $CD69^+/CD57^+/CD28^{null} CD4^+$ T-cell percentage, as well as with CRP values greater than 1 mg/L and shorter telomere length. Lower post-BD FEV_1 percent-predicted was also associated with elevated $CD69^+/CD57^+/CD28^{null} CD4^+$, as well as elevated IL-6 and CRP > 1 mg/L. As with DL_{CO} , higher percentages of $CD57^+/CD28^{null} CD8^+$ T-cells correlated with better post-BD FEV_1 (Table 3). Higher odds of airflow obstruction (FEV_1/FVC ratio < 0.7) post-BD were found in association with CRP levels > 1 mg/L and with shorter telomere length. Lower odds of both pre-BD and post-BD obstruction were associated with higher $CD57^+/CD28^{null} CD8^+$ T-cell percentages (Table 4).

Virally-suppressed subgroup

Although all multivariable analyses assessed for covariates related to HIV infection (including duration of HIV, viral load, CD4⁺ T-cell count, and use of ART), it is possible that persistent viremia may have an undetected contribution confounding the relationships between inflammatory and senescent biomarkers and pulmonary function. *Post-hoc* subgroup analyses restricted to participants with undetectable HIV viral load (n = 100) were performed for the significant findings reported in Tables 3 and 4. In the virally-suppressed group, CD25⁺/CD4⁺, CD25⁺/CD8⁺, and CRP retained association with worse DL_{CO} (dichotomous and continuous measure), and CD57⁺/CD28^{null} CD8⁺, CRP, and telomere length retained similar associations with airflow obstruction. CD69⁺/CD57⁺/CD28^{null} CD4⁺ cell percentages and IL-6 did not retain significant associations with PFT outcomes in the virally-suppressed group (Supplemental Tables 1A and 1B). Whether this finding points to a mediation of effects by active viremia or is simply a limitation of sample size cannot be addressed.

Biomarker correlations

Associations were demonstrated between several of the biomarkers that associated with pulmonary function in multivariable models. CD25⁺/CD8⁺ and CD25⁺/CD4⁺ percentages were each modestly correlated with IL-6 (Table 5). Neither CD25⁺ variable was associated with PBMC telomere length or with CD69⁺/CD57⁺/CD28^{null} CD4⁺ percentages. Higher IL-6 levels were associated with higher CRP levels and also with shorter PBMC telomere length (Table 5).

DISCUSSION

This study is the first to relate immune activation, systemic inflammation, and T-cell senescence with pulmonary dysfunction in HIV-infected persons. Our data show that worse airflow obstruction and lower DL_{CO} are associated with increased immune activation and inflammation. Cellular senescence was also associated in a complex manner with diffusing impairment and airflow obstruction. These relationships were independent of other influences on lung function such as cigarette smoking and previous pneumonia.

We found that CD25 and CD69, classical markers of activated T-cells, were each associated with PFT abnormalities. CD25 was expressed equivalently on CD4⁺ and CD8⁺ T-cells, and was associated with diffusing impairment as both a continuous and dichotomous measure. When associations between cellular and humoral biomarkers were evaluated, CD25 expression was found to positively correlate with IL-6, which was also associated with both diffusing impairment and airflow obstruction in this cohort. A second marker of early activation, CD69, was associated with both lower diffusing capacity and lower FEV₁ percent-predicted, but only when expressed in concert with CD57⁺/CD28^{null}, representing a senescent, activated phenotype. These data support a relationship between immune activation, systemic inflammation, and pulmonary dysfunction in HIV. To our knowledge, this is the first report integrating cell activation and senescence markers to evaluate T-cell phenotypes in HIV-associated chronic disease. It is important to note that CD25⁺ T-cells, particularly CD4⁺ cells, may represent either an activated or a regulatory

phenotype. As we did not perform intracellular Foxp3⁺ staining, the percentage of CD25⁺ CD4⁺ cells which are regulatory T-cells is unknown. While the direct association with the inflammatory marker IL-6 and the expected finding of association with pulmonary dysfunction suggests a classical activation phenotype, these findings will require confirmation in future cohorts. We did not find associations in this cohort between CD38, another marker of T-cell activation, and pulmonary function outcomes. No prior study has evaluated CD38 in HIV-associated COPD, and reports examining CD38⁺ T-cell percentages and HIV-associated cardiovascular disease have been conflicting. One cross-sectional study reported association between CD38⁺HLA-DR⁺ T-cells and carotid disease[34,35], while others have not found such associations[47]. Detection of CD38⁺ T-cell associations in the current study may have been limited by sample size or lack of measurement of HLA-DR⁺ co-expression. Nevertheless, our data correlating immune activation markers CD25 and CD69 with poorer PFT outcomes suggest these may be important in the development of long-term pathologic sequelae of chronic HIV infection.

Of the humoral inflammatory markers investigated, both IL-6 and CRP were increased in individuals with worse pulmonary function. Higher plasma IL-6 levels were associated with both diffusing impairment and lower post-bronchodilator FEV₁, and CRP was related to all measures of pulmonary dysfunction. These inflammatory molecules are likely to be of pathogenic significance in persons chronically infected with HIV. CRP is persistently elevated in chronic HIV infection (including among virally-suppressed persons[48]) and is associated with disease progression[49]. Although links with HIV-associated COPD have not previously been established, other HIV-associated chronic diseases, including cardiovascular disease, are related to elevated IL-6[39] and CRP[39,50]. Similarly, IL-6 and CRP are associated with COPD diagnosis[40,51] and outcomes[40,52], and CRP has been linked to exacerbation risk[53] and presence of comorbidities[54] in the HIV-uninfected population.

In our HIV-infected cohort, IL-6 and CRP levels were strongly related, suggesting a systemic inflammatory phenotype in some participants. A recent study of HIV-uninfected COPD patients found persistent systemic inflammation is associated with COPD outcomes, but not with traditional markers of disease severity, suggesting that non-pulmonary mediators driving systemic inflammation may be contributing to disease outcomes[40]. Among HIV-infected persons, immune activation or other HIV-specific factors may promote this systemic inflammation that can worsen COPD.

Immune senescence is an area of interest in HIV and its chronic comorbidities[55,56], but it has not been investigated in HIV-associated COPD. We found that measures of senescence demonstrated a complex relationship to pulmonary function, with differing results obtained for PBMC telomere length versus T-cell surface markers (CD57⁺/CD28^{null}). Additionally, in our cohort, there were no correlations between PBMC telomere length and T-cell CD57⁺/CD28^{null} percentages. While the flow cytometry assays of senescence were limited to selected CD4⁺ and CD8⁺ T-cells, telomere measurements were performed on all leukocytes and therefore represent a more heterogeneous population of cells. More importantly, as discussed below, these two assays may capture different features and mechanisms of “senescence” and may not represent the same functional deficit.

Shorter PBMC telomere length (a known bioindicator of replicative senescence in human somatic cells[57]) was associated with lower pre-bronchodilator FEV₁ and higher odds of post-bronchodilator airflow obstruction. This relationship has not been previously reported in HIV-associated COPD. In the HIV-uninfected population, PBMC telomere shortening has been independently associated with COPD presence and severity in multiple studies[58–61]. Telomere shortening is also a well-described feature of HIV infection[62–64], independent of the degree of immune suppression[63]. The mechanisms and direction of the relationships among HIV, PBMC telomere shortening, and COPD are not yet clear. Considering the natural course of HIV, the overall telomere shortening of the CD8⁺, granulocyte, monocyte, and B cell compartments[65–68] could be a consequence of either replicative senescence due to repeated clonal expansion of immune cells due to HIV and its co-infections or skewed expansion of any or all of these cells to occupy the immunological space “vacated” by CD4⁺ cells. Whether there are infection-associated factors that directly destabilize telomere structure and/or elicit overall DNA instability in leukocytes in HIV remains to be examined.

Interestingly, despite the observed association of airflow obstruction and PBMC telomere shortening, our data also show that higher percentages of T-cells displaying phenotypic markers of senescence (CD57⁺/CD28^{null}) were associated with lower risk of diffusing impairment and with lack of airflow obstruction. These findings are consistent with emerging themes related to the complexity of the biological function of senescent cells. In the immune system, it is clear that the irreversible loss of CD28 is the best known indicator of T-cell aging[69], and the frequency of CD28^{null} CD4⁺ T-cells has been associated with severity and chronicity of many chronic inflammatory diseases in the young[70]. CD28^{null} T-cells, however, are functionally heterogeneous, due to the induction of cell surface receptors that can either have detrimental or beneficial function. For example, *de novo* expression of CD31 on CD28^{null} CD8⁺ T-cells and CD56 on CD28^{null} CD4⁺ T-cells is associated with the oligoarticular subtype of juvenile idiopathic arthritis[72] and with idiopathic lung disease in young adults with rheumatoid arthritis[73], respectively. In contrast, studies of successfully aging octo/nonagenarians show expression of various NK-related receptors on CD28^{null} CD4⁺ and CD8⁺ T-cells, with triggering of such receptors leading to production of immune protective cytokines such as IL-2 and interferon γ [20,74]. In the present study, our data show that poorer pulmonary function is correlated with prevalence of CD69⁺/CD57⁺/CD28^{null} CD4⁺ T-cell subsets, likely representing T-cells that have adverse effects on lung function. Conversely, better pulmonary function is correlated with the broader CD57⁺/CD28^{null} CD8⁺ T-cell population, suggesting that there might a particular senescent CD8⁺ T-cell subset that has beneficial immune effector function. As recently demonstrated in a study examining the CD57⁺ compartment of CD28^{null} T-cells in HIV-infected persons (and in contrast to findings in older non-HIV infected adults), lower percentages of CD57⁺ cells within the CD28^{null} compartment associate with higher circulating markers of innate immune activation and worse mortality outcomes[71]. The lack of expression of CD57⁺ on CD28^{null} cells suggests a failure of terminal differentiation and successful proliferative capacity in response to infectious triggers. The CD57^{null}/CD28^{null} cells may therefore mark persons at increased risk for persistent immune activation and its organ-specific downstream effects. The prognostic

relevance of this particular T-cell phenotype has yet to be determined, and further evaluation in longitudinal cohorts will be revealing. A final alternative is that these cells may in fact be deleterious, but are recruited to the lung in individuals with COPD, resulting in decreased peripheral numbers. Indeed, lung CD8⁺ T-cells have been associated with COPD development in animal and human studies[75,76] and are thought to contribute to lung destruction via elaboration of inflammatory mediators[76–78]. Of interest, therefore, are further studies to determine the subsets of CD8⁺ T-cells in the circulation and/or in the lung which may have beneficial or detrimental effector functions. Further studies characterizing the immune components of the pulmonary compartment in HIV-infected persons will improve our understanding of T-cell dynamics and COPD in this population.

This study has several limitations. First, due to the cross-sectional design, we were able to capture associations, but cannot describe predictive characteristics. Further studies designed to follow progression of pulmonary function over time will be better able to address cause-effect relationships. Additionally, participants enrolled in the study have multiple comorbidities and exposures that may impact both pulmonary function and levels of circulating biomarkers, making precise estimates of associations difficult despite efforts to adjust for confounders. In particular, many participants were current or former smokers, and mechanisms of pulmonary dysfunction in these groups may be different. Nonetheless, we feel that the sample in this study accurately represents the current US HIV-infected population, and that the biomarker findings are relevant to the population's disease states. Finally, we may not have included all immune activation/senescence markers or inflammatory cytokines that are important in HIV-associated COPD, but we feel that the data presented form groundwork for the further study of immune-inflammatory relationships in association with pulmonary outcomes.

In summary, the pathogenesis of pulmonary complications of chronic HIV disease in the ART era is of significant interest, but remains relatively uncharted territory. Characterization of immune and inflammatory factors associated with adverse pulmonary outcomes is an important step in determining mechanisms of development and progression of HIV-associated COPD. This study is the first to demonstrate that HIV-associated pulmonary dysfunction is characterized by peripheral T-cell activation and inflammation and is linked to features of cell senescence. Further studies are needed to evaluate the underpinnings of telomere shortening in HIV, to examine circulating and local subsets of activated T-cells and CD57⁺/CD28^{null} CD8⁺ T-cells, and to elucidate the specific triggers and consequences of immune and inflammatory pathways, which may provide rationale for future targeted therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of participants (n = 147)

Male, n (%)	98 (66.7)
Race/ethnicity	
– Black, n (%)	82 (55.8)
– Hispanic, n (%)	5 (3.4)
Age (years), mean (SD)	45.6 (9.4)
Smoking history (pack years), median (IQR)	10 (1.4–22)
Smoking history, categorized	
– Current smoker, n (%)	84 (57.1)
– Ever smoker (>100 cigarettes), n (%)	122 (83.0)
Injection drug use	
– Current, n (%)	7 (4.8)
– Ever, n (%)	30 (20.4)
Using ART, n (%)	129 (87.8)
Viral load undetectable (* n=146), n (%)	100 (68.5)
CD4 count (* n=146), med (IQR)	535 (333–747)
CD4 count < 200 cells/μl (* n=146), n (%)	15 (10.3)
Prior pneumonia history	
– Bacterial, n (%)	68 (46.3)
– <i>Pneumocystis</i> (* n=133), n (%)	13 (9.8)
Hepatitis C RNA positive (* n=135), n (%)	25 (18.5)
FEV ₁ %predicted, pre-BD (* n=146), mean (SD)	0.90 (0.19)
FEV ₁ %predicted, post-BD (* n=146), mean (SD)	0.94 (0.18)
FEV ₁ /FVC ratio, pre-BD (* n=146), mean (SD)	0.74 (0.09)
FEV ₁ /FVC ratio, post-BD (* n=146), mean (SD)	0.77 (0.10)
DL _{CO} %predicted, mean (SD)	0.67 (0.14)
FEV ₁ /FVC ratio < 70% (* n=146)	
– Pre-BD n (%)	42 (28.8)
– Post-BD, n (%)	31 (21.2)
DL _{CO} < 60 %predicted, n (%)	50 (34.0)

ART: antiretroviral therapy, BD: bronchodilator, DLCO: diffusing capacity for carbon monoxide, FEV₁: forced expiratory volume in one second, FVC: forced vital capacity, IQR: interquartile range, SD: standard deviation

* if complete data for all 147 participants not available, alternative n is noted within the table

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Table 2

Distributions of circulating biomarkers.

Variable	number with data	Median (IQR)
CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺	88	2.4 (0.6 – 9.0)
CD25 ⁺ , % of CD4 ⁺	88	3.8 (2.1 – 7.4)
CD38 ⁺ , % of CD4 ⁺	88	6.7 (3.9 – 11.1)
CD69 ⁺ , % of CD4 ⁺	88	15.4 (4.9 – 30.6)
CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺	48	0.1 (0.0 – 0.8)
CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺	48	1.6 (0.8 – 3.5)
CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺	48	2.2 (0.6 – 5.7)
CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺	88	35.6 (21.9 – 42.4)
CD25 ⁺ , % of CD8 ⁺	88	0.3 (0.1 – 1.0)
CD38 ⁺ , % of CD8 ⁺	88	4.0 (1.4 – 8.0)
CD69 ⁺ , % of CD8 ⁺	88	7.4 (3.0 – 14.5)
CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺	88	0.1 (0.0 – 0.3)
CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺	88	2.4 (0.8 – 6.5)
CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺	88	4.1 (1.4 – 8.6)
Telomere length (relative ratio)	114	0.8 (0.7 – 1.0)
IL-8, plasma (pg/mL)	123	9.7 (8.0 – 11.8)
IL-6, plasma (pg/mL)	123	2.0 (1.1 – 3.3)
hsCRP, plasma (mg/L)	136	1.6 (0.1 – 6.8)

hsCRP: high sensitivity C-reactive protein, IL: interleukin

Table 3

Coefficients and p-values for relationships between tested biomarkers and each PFT outcome in individual multivariable linear regression models. Biomarkers are displayed in order of significance of relationship for each PFT outcome. Significant covariates are shown in column 5 for models in which the biomarker of interest displayed a significant relationship.

PFT outcome	Model Biomarker Variable	Coef	p-value	Covariates
DL _{CO} % predicted	CRP, plasma > 1mg/L (136) *	-0.068	0.001	History of <i>Pneumocystis</i> pneumonia, History of injection drug use, BMI
	IL-6, plasma (pg/mL), Q4 (122) *	-0.075	0.005	Pack-years smoked, History of <i>Pneumocystis</i> pneumonia, BMI
	CD25 ⁺ , % of CD4 ⁺ , Q4 (87) *	-0.082	0.006	Pack-years smoked, History of <i>Pneumocystis</i> pneumonia, BMI
	CD25 ⁺ , % of CD8 ⁺ , Q4 (88) *	-0.073	0.02	History of <i>Pneumocystis</i> pneumonia, BMI
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48) *	-0.086	0.03	History of <i>Pneumocystis</i> pneumonia
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (87)	-0.002	0.13	n/a
	CD38 ⁺ , % of CD4 ⁺ , Q4 (88)	-0.042	0.18	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (87)	-0.041	0.19	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (87)	-0.038	0.23	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (88)	-0.036	0.25	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (88)	-0.029	0.35	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	-0.033	0.44	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (87)	0.023	0.47	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (88)	-0.022	0.48	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (88)	0.001	0.54	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.025	0.56	n/a
	Telomere length (relative ratio) (114)	0.011	0.81	n/a
	IL-8, plasma (pg/mL), Q4 (123)	-0.004	0.89	n/a
FEV ₁ % predicted, pre-BD	CRP, plasma > 1mg/L (135) *	-0.106	<0.001	History of <i>Pneumocystis</i> pneumonia
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48) *	-0.150	0.01	BMI
	Telomere length (relative ratio) (113) *	0.138	0.05	None
	CD25 ⁺ , % of CD4 ⁺ , Q4 (86)	-0.084	0.07	n/a
	IL-6, plasma (pg/mL), Q4 (123)	-0.063	0.08	n/a
	IL-8, plasma (pg/mL), Q4 (123)	-0.056	0.12	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (86)	0.002	0.24	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	-0.050	0.28	n/a
	CD25 ⁺ , % of CD8 ⁺ , Q4 (86)	-0.048	0.29	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (86)	0.046	0.31	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (86)	-0.040	0.37	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (86)	-0.033	0.47	n/a

PFT outcome	Model Biomarker Variable	Coef	p-value	Covariates
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	−0.001	0.51	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (86)	−0.031	0.51	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	−0.006	0.89	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.005	0.94	n/a
	CD38 ⁺ , % of CD4 ⁺ , Q4 (86)	0.002	0.96	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.001	0.99	n/a
FEV ₁ % predicted, post-BD	CRP, plasma > 1mg/L (135)*	−0.093	0.001	History of <i>Pneumocystis</i> pneumonia
	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (86)*	0.002	0.03	Pack-years smoked, History of <i>Pneumocystis</i> pneumonia, BMI
	IL-6, plasma (pg/mL), Q4 (123)*	−0.074	0.03	History of <i>Pneumocystis</i> pneumonia
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)*	−0.118	0.04	BMI
	CD25 ⁺ , % of CD4 ⁺ , Q4 (86)	−0.080	0.06	n/a
	Telomere length (relative ratio) (113)	0.109	0.09	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (86)	0.058	0.16	n/a
	IL-8, plasma (pg/mL), Q4 (123)	−0.042	0.23	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.061	0.34	n/a
	CD25 ⁺ , % of CD8 ⁺ , Q4 (86)	−0.037	0.37	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	−0.001	0.42	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (86)	−0.020	0.64	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	0.013	0.76	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	−0.013	0.77	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (86)	0.010	0.81	n/a
	CD38 ⁺ , % of CD4 ⁺ , Q4 (86)	0.004	0.92	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.005	0.94	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (86)	−0.003	0.95	n/a

BD: bronchodilator, BMI: body mass index, CRP: C-reactive protein, DLCO: diffusing capacity for carbon monoxide, FEV₁: forced expiratory volume in one second, FVC: forced vital capacity, IL: interleukin, Q4: fourth quartile (>75th percentile)

* indicates significance at an alpha of 0.05

Each biomarker was modeled individually in relationship to PFT outcomes with relevant covariates included.

Table 4

Odds ratios (OR) and p-values for relationships between each tested biomarker and each PFT outcome in individual multivariable logistic regression models. Biomarkers are displayed in order of significance of relationship for each PFT outcome. Significant covariates are shown in column 5 for models in which the biomarker of interest displayed a significant relationship

PFT outcome	Model Biomarker Variable	OR	p-value	Covariates
DL _{CO} < 60% predicted	IL-6, plasma (pg/mL), Q4 (122)*	3.758	0.006	Pack-years smoked, BMI
	CD25 ⁺ , % of CD8 ⁺ , Q4 (88)*	4.246	0.01	History of bacterial pneumonia, History of injection drug use
	CRP, plasma > 1mg/L (136)*	2.557	0.02	History of injection drug use
	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (88)*	0.962	0.03	History of bacterial pneumonia
	CD25 ⁺ , % of CD4 ⁺ , Q4 (88)*	2.924	0.05	History of bacterial pneumonia
	CD38 ⁺ , % of CD4 ⁺ , Q4 (88)	2.401	0.09	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (88)	1.891	0.23	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.354	0.29	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (88)	0.592	0.38	n/a
	IL-8, plasma (pg/mL), Q4 (122)	0.694	0.44	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (88)	1.011	0.52	n/a
	Telomere length (relative ratio) (114)	1.723	0.54	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	1.677	0.58	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (88)	1.237	0.70	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (88)	1.130	0.83	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (88)	1.113	0.85	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.868	0.88	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (88)	0.982	0.97	n/a
FEV ₁ /FVC < 70%, pre-BD	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (86)*	0.926	0.003	Pack-years smoked, Age (per year)
	IL-6, plasma (pg/mL), Q4 (122)	2.545	0.06	n/a
	CD38 ⁺ , % of CD4 ⁺ , Q4 (87)	3.031	0.08	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (86)	3.245	0.08	n/a
	CRP, plasma > 1mg/L (135)	2.085	0.09	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (87)	0.323	0.10	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	3.353	0.18	n/a
	Telomere length (relative ratio) (113)	2.861	0.33	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (87)	0.626	0.46	n/a
	CD25 ⁺ , % of CD8 ⁺ , Q4 (86)	1.568	0.49	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	1.012	0.52	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.600	0.54	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (86)	0.711	0.63	n/a

PFT outcome	Model Biomarker Variable	OR	p-value	Covariates
	IL-8, plasma (pg/mL), Q4 (122)	1.153	0.77	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (86)	1.188	0.79	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	1.175	0.84	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	0.884	0.86	n/a
	CD25 ⁺ , % of CD4 ⁺ , Q4 (86)	1.093	0.90	n/a
FEV ₁ /FVC < 70%, post-BD	CD57 ⁺ /CD28 ^{null} , % of CD8 ⁺ (86) *	0.929	0.005	Pack-years smoked, Age (per year)
	Telomere length (relative ratio) (113) *	0.031	0.01	Pack-years smoked, BMI
	CRP, plasma > 1mg/L (134) *	3.086	0.04	Pack-years smoked, Age (per year), CD4 count, current
	IL-6, plasma (pg/mL), Q4 (122)	1.992	0.19	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	0.383	0.24	n/a
	CD25 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	0.318	0.31	n/a
	CD25 ⁺ , % of CD8 ⁺ , Q4 (86)	0.471	0.34	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (47)	0.373	0.37	n/a
	CD57 ⁺ /CD28 ^{null} , % of CD4 ⁺ (86)	0.520	0.41	n/a
	CD38 ⁺ , % of CD4 ⁺ , Q4 (86)	1.624	0.52	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	1.011	0.60	n/a
	CD69 ⁺ , % of CD4 ⁺ , Q4 (86)	1.438	0.62	n/a
	CD69 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD8 ⁺ , Q4 (86)	1.412	0.64	n/a
	IL-8, plasma (pg/mL), Q4 (122)	1.276	0.64	n/a
	CD38 ⁺ , % of CD57 ⁺ /CD28 ^{null} CD4 ⁺ , Q4 (48)	1.314	0.78	n/a
	CD38 ⁺ , % of CD8 ⁺ , Q4 (86)	1.154	0.84	n/a
	CD25 ⁺ , % of CD4 ⁺ , Q4 (86)	1.053	0.94	n/a
	CD69 ⁺ , % of CD8 ⁺ , Q4 (86)	1.052	0.95	n/a

BD: bronchodilator, BMI: body mass index, CRP: C-reactive protein, DLCO: diffusing capacity for carbon monoxide, FEV₁: forced expiratory volume in one second, FVC: forced vital capacity, IL: interleukin, Q4: fourth quartile (>75th percentile)

* indicates significance at an alpha of 0.05.

Each biomarker was modeled individually in relationship to PFT outcomes with relevant covariates included.

Table 5

Correlations among the biomarkers associated with pulmonary function.

	CD25 ⁺ , % of CD8 ⁺	CD69 ⁺ , % of senescent CD4 ⁺	IL-6	CRP	Telomere length	Senescent CD8 ⁺
CD25 ⁺ , % of CD4 ⁺	r = 0.714 p < 0.0001 *	r = -0.034 p = 0.82	r = 0.255 p = 0.03 *	rho = 0.093 p = 0.42	r = 0.126 p = 0.32	r = 0.078 p = 0.47
CD25 ⁺ , % of CD8 ⁺		r = 0.188 p = 0.20	r = 0.288 p = 0.01 *	rho = 0.057 p = 0.62	r = 0.057 p = 0.65	r = -0.176 p = 0.10
CD69 ⁺ , % of senescent CD4 ⁺			r = 0.044 p = 0.79	rho = 0.106 p = 0.50	r = -0.111 p = 0.52	r = -0.098 p = 0.51
IL-6				rho = 0.362 p = 0.0001 *	r = -0.322 p = 0.002 *	r = -0.040 p = 0.74
CRP					rho = -0.092 p = 0.33	rho = 0.083 p = 0.47
Telomere length						r = -0.064 p = 0.61

Variables log-transformed with exception of CRP, telomere length, and senescent CD8⁺. Correlation coefficients reported as r for Pearson correlations or rho for Spearman correlations.

* indicates p-value at significance level <0.05.

CRP: C-reactive protein, IL: interleukin, senescent: CD28^{null}/CD57⁺