The Journal of Infectious Diseases
MAJOR ARTICLE

Persistent Increased Plasmacytoid Dendritic Cells and Inflammation in People With HIV Years After Tuberculosis

Maureen Ward,[1] Paul Zumbo,[2] Yvetot Joseph,[3] Alexandra Apollon,[3] Alicia Alonso,[1] Doron Betel,[1,2,4] Daniel W. Fitzgerald,[1] Jean W. Pape,[1,3] and Kathryn M. Dupnik[1]

[1]Department of Medicine, Weill Cornell Medicine, New York, New York, USA; [2]Applied Bioinformatics Core, Weill Cornell Medicine, New York, New York, USA; [3]GHESKIO, Port au Prince, Haiti; and [4]Institute for Computational Biomedicine, Weill Cornell Medicine, New York, New York, USA

Background. People living with human immunodeficiency virus (HIV-1) who have a history of cured tuberculosis have worse outcomes, including increased all-cause mortality rate and risk of recurrent tuberculosis. We hypothesized that persistent and global immune deficits could contribute to these outcomes in people with a history of tuberculosis.

Methods. We completed FLEX Cellular Indexing of Transcriptomes and Epitopes by Sequencing (FLEX-CITE-Seq) of peripheral blood mononuclear cells of people living with HIV with (n = 6) or without (n = 3) a history of tuberculosis at GHESKIO Centers in Haiti. We subtyped dendritic cells using flow cytometry and quantitated cytokines on an expanded cohort (n = 29) to confirm FLEX-CITE-Seq findings.

Results. Cell types with significantly differential levels of expression for >40 genes all had overrepresentation of a tumor necrosis factor (TNF)–mediated pathway. In an expanded cohort of 29 people living with HIV, we found a larger percentage of plasmacytoid dendritic cells by flow cytometry and increased plasma interleukin 6, 12p70, 15, and 2, interferon α, and TNF levels in the tuberculosis history group (n = 18) than in the group with no history of tuberculosis (n = 11).

Conclusions. A proinflammatory milieu and immune cell gene expression changes mediated by TNF persist in people living with HIV even years after tuberculosis is cured. It remains to be determined whether the differences are preexisting risk factors or are established during the natural history of HIV and tuberculosis infections.

Keywords. tuberculosis; HIV; dendritic cells; TNF; single-cell RNA-Seq.

People living with human immunodeficiency virus (HIV-1) who have a history of tuberculosis have worse clinical outcomes, including an increased all-cause mortality rate, than those with HIV who have never had tuberculosis [1–4]. The risk of death is greatest in people with advanced HIV and low $CD4^+$ T-cell counts at the time of tuberculosis and HIV diagnoses [5, 6]. The increased risk of death persists even when accounting for tuberculosis treatment, antiretroviral therapy, and isoniazid prophylaxis. In a study in Haiti, tuberculosis was the only predictor of increased mortality risk in a multivariate analysis, with a hazard ratio of 2.78 (95% confidence interval, 1.61–4.79) [1]. After exclusion of early deaths, the 5-year mortality rate was 14.2% in the tuberculosis cohort and 6.1% in the nontuberculosis cohort (log-rank $P < 0.001$) [1]. Even when tuberculosis is diagnosed, treated, and cured —and even when potential confounders are controlled for— people with tuberculosis-HIV coinfection have an increased risk of future death.

We hypothesized that persistent immune aberrations contribute to worse outcomes in people with history of tuberculosis. To assess the impact of history of active pulmonary tuberculosis on immune cell populations in the peripheral blood of people living with HIV, we began with single-cell RNA-Seq (scRNA-Seq) of peripheral blood mononuclear cells (PBMCs). The scRNA-Seq approach in studies of HIV pathogenesis [7, 8] and tuberculosis monoinfection [9–13] is reviewed elsewhere. Published single-cell transcriptomic studies of coinfection have focused on small numbers of people with active tuberculosis [14] or cells infected with *Mycobacterium tuberculosis* (*Mtb*) ex vivo [15].

For the current study, we applied FLEX scRNA-Seq technology to profile fixed human cells [16] with Cellular Indexing of

Received 11 August 2025; accepted 20 September 2025; published online 7 October 2025
Presented in part: International Union Against Tuberculosis and Lung Disease meeting, Bali, Indonesia, November 14, 2024; International AIDS Society annual meeting, Kigali, Rwanda, July 16, 2025.

Correspondence: Kathryn M. Dupnik, MD, Department of Medicine, Weill Cornell Medicine, 420 E 70th St, Ste LH-455, New York, NY 10021 (kad9040@med.cornell.edu).

The Journal of Infectious Diseases® 2026;233:e902–12

https://doi.org/10.1093/infdis/jiaf499

Transcriptomes and Epitopes by Sequencing (CITE-Seq) for surface protein labeling of fixed human cells [17]. To demonstrate the proinflammatory environment in people living with HIV with history of tuberculosis, we pursued biologic confirmation of a cell type whose differential gene expression profile could plausibly mediate the immune response to *Mtb* during HIV coinfection, using a complementary cytokine analysis.

METHODS

Study Population

The tuberculosis rate in Haiti is 154 new cases per 100 000 people [18], of whom 15% also have HIV [18]. The nongovernmental organization Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections (with the French acronym of GHESKIO) in Port au Prince, Haiti is the largest provider of HIV and tuberculosis care in the Americas. Samples for this study were obtained from a previously described study of tuberculosis recurrence at GHESKIO [19].

People living with HIV with history of recurrent or a single episode of tuberculosis or no prior tuberculosis were enrolled 1:1:1 for a single study visit. Active pulmonary tuberculosis was bacteriologically confirmed with TB GeneXpert assay or culture. Tuberculosis episodes were concurrent with or after the diagnosis of HIV, and tuberculosis treatment had been completed for ≥6 months prior to study enrollment. People with both detectable viral load and plasma p24 antigen, as described elsewhere [19], were excluded from this study. Tuberculosis case patients and no-tuberculosis controls were matched for sex, age, and time since diagnosis of HIV.

PBMCs

PBMCs were isolated between December 2019 and October 2023 using density gradient separation (Ficoll-Hypaque; GE Healthcare) with 5×10^6 PBMC aliquots frozen in fetal bovine serum and dimethyl sulfoxide. PBMCs were identified with a unique specimen identifier that did not correspond to tuberculosis status.

Fixed RNA Profiling With FLEX-CITE-Seq of PBMCs

PBMCs were prepared for sequencing using the 10x Genomics protocols CG000529 Rev A and CG000478 Rev A. After thawing, live cells were negatively selected (EasySep Dead Cell Removal; Stem Cell Technologies). Cell surface immune lineage markers were labeled using the TotalSeq-B Human TBNK Cocktail (BioLegend 399902). Labeled live cells were fixed and frozen (10x Genomics; PN-1000414). Library preparation, feature barcoding, and sequencing for Chromium Single Cell Gene Expression FLEX (10x Genomics), a hybridization-based platform for scRNA-Seq of fixed cells, were performed according to the manufacturer's instructions. Gene expression and CITE-Seq libraries were pooled and sequenced on 1 lane of an Illumina NovaSeq 6000 sequencer. Matched cases and controls were prepared and sequenced together.

Analysis of FLEX scRNA-Seq and CITE-Seq Data

Raw sequencing reads were processed using the Cell Ranger (10x Genomics) multipipeline (version 7.1.0) to demultiplex, align, and generate feature-barcode matrices with associated epitope data. The GRCh38 human genome (Build 2020-A; 10x Genomics) and the Chromium Human Transcriptome Probe Set (version 1.0.1) were used as references. Cells with ≥10% mitochondrial reads [20] were removed. Cells were considered outliers if they were >2.5 SDs away from the mean values for either unique molecular identifier (UMI) count or detected RNA features. Cells inferred to be doublets by the scDblFinder package (version 1.16.0) [21] were removed. Cells were integrated across donors with anchor-based canonical correlation analysis integration and then subjected to UMAP dimensionality reduction and clustering using Seurat software (version 4.2.0) [22]. Cells were clustered using both transcriptome and cell-surface protein expression data with a weighted nearest neighbors approach. Cells were annotated with SingleR software (version 2.4.0) [23] using the Human Primary Cell Atlas reference dataset [24] and with Seurat Azimuth reference transfer using its composite PBMC reference [25]. Cell type annotations were harmonized between the references via manual inspection of canonical gene expression. As a secondary analysis, we assigned cells to the 6 dendritic cell (DC) clusters (DC1–DC6) described by Villani et al [26]. We applied the Seurat AddModuleScore function to the gene signatures of each DC cluster and calculated the activity of the hallmark TNF signaling via NF-κB gene set in each cell type.

DC Phenotyping

To quantitate DC subsets phenotypically in 5×10^6 PBMCs, we thawed, washed, and stained with a fluorescently labeled antibody panel (Supplementary Table 1) to distinguish Type 1 conventional (cDC1), Type 2 conventional (cDC2), and plasmacytoid DCs (pDCs). Flow cytometry (fluorescence-activated cell sorting [FACS]) data were collected with a Fortessa cytometer using FACS DIVA software and analyzed using FlowJo software (Becton-Dickinson). Compensation beads, full-minus-1 panels, and cells from ≥1 person from both tuberculosis and no-tuberculosis groups were included in each FACS run. The gating strategy was optimized from published studies of FACS DC phenotyping [27–29] (Supplementary Figure 1). DCs were enumerated from the single nucleated cell lineage negative ($CD19^-$, $CD3^-$, $CD56^-$, $CD15^-$, $CD16^-$, and $CD14^-$) $HLA\text{-}DR^+CD11c^+$ cell population. DC1 cells were $CD141^+CD1c^-$. DC2 were $CD1c^+$ and $CD141^-$. pDCs were $CD303^+$.

Cytokine Assays

To assess for plasma interferon (IFN) α and additional cytokines, we used an 18-plex Th1/Th2 panel (ProcartaPlex 1C;

EPX180-12172-901; Thermo Fisher) on undiluted previously unthawed plasma stored at −80°C and run in duplicate. Absorbance raw data were recorded on a MAGPIX with xPONENT software (version 4.3; Luminex). Standard curves were generated and sample concentrations calculated using the ProcartaPlex Analysis App (Thermo Fisher).

Statistics

Power Calculation for CITE-Seq Data

We used the scPower framework [30] to consider power for scRNA-Seq [31]. For $CD4^+$ T cells in PBMCs at a frequency of 20%, a 2:1 case-control ratio, a total sample size of 9, and output data for 6000–10 000 cells, we had >80% power to detect significantly differentially expressed genes.

Statistical Analysis of CITE-Seq Data

Differential expression analysis was performed using a pseudobulk approach [32, 33]. For each cell type, gene expression counts across cells within a biological replicate were aggregated by summing using the R Libra software package (version 1.0.0) [34]. We used DESeq2 software (version 1.42.0) Wald tests [35] to compare gene expression between tuberculosis and no-tuberculosis groups. Genes were considered differentially expressed at $|\log_2 \text{fold change (FC)}| \geq 0.25$ and adjusted P (P_{adj}) ≤ 0.05. For immune cell subsets with ≥40 genes differentially expressed between tuberculosis and no-tuberculosis groups, the differentially expressed genes were input for Gene Ontology (GO) biological process enrichment pathways [36] using clusterProfiler software (version 4.10.1) [37] and for Gene Set Enrichment Analysis (GSEA) hallmark pathways with fgsea software (version 1.28.0) [38] against MSigDB hallmark gene sets [39].

Statistical Analysis of DC Data

Sample size for the DC studies was calculated based on preliminary FACS studies on the 9 participants who had FLEX CITE-Seq. We used the power command in Stata to calculate the sample size for the following parameters: α = 0.05; power, 0.80; case-control ratio, 2.0; means, 1.12% and 3.4%; and SD, 2.0%. For the DC analysis, we compared tuberculosis versus no-tuberculosis groups using Mann-Whitney tests at α = .05. When outliers were present, additional sensitivity analysis excluded outliers to confirm the association. We used tobit regression analysis with history of tuberculosis as the independent variable to account for left censoring and to test for sex as a covariate.

Statistical Analysis of Cytokine Data

For the cytokine analysis, we imputed a value of 0 when absorbance was less than the limit of calculation. Cytokines for which more than half of samples were less than the limit of calculation did not continue to statistical analysis. The primary analysis was for IFN-α. Remaining cytokines in the panel were analyzed as a secondary analysis with Bonferroni correction for multiple comparisons. When indicated, we included tobit regression analysis with history of tuberculosis as the independent variable.

Sex as a Biological Variable

We matched case participants (with a history of tuberculosis) with controls (with no history of tuberculosis) by sex. We included sex as a covariate in tobit regression of DC populations and cytokine levels.

Study Approval

This study was approved by the institutional review boards at Weill Cornell Medicine (protocol 1612017803) and GHESKIO. All study participants provided written informed consent with a 2-step consent protocol at GHESKIO [40]. Human research guidelines of the US Department of Health and Human Services, Weill Cornell Medicine, and GHESKIO were followed and in accordance with the Helsinki Declaration.

Data Availability

scRNA-Seq data will be available in the National Center for Biotechnology Information's GEO system. Raw data associated with this article are archived in Weill Cornell Medicine's research data repository.

RESULTS

We generated FLEX-Seq and CITE-Seq data from PBMCs of 6 people living with HIV with a history of tuberculosis and 3 with no tuberculosis history (Table 1 and Supplementary Table 2). Gene expression data corresponded to immune cell phenotypes identified by cell surface antibody staining. These data were clustered using Seurat software and projected to the Human Primary Cell Atlas (Figure 1*A*) and Azimuth PBMC (Figure 1*B*) references as expected. When tuberculosis history

Table 1. Characteristics of Participants With Peripheral Blood Mononuclear Cells Included in FLEX Cellular Indexing of Transcriptomes and Epitopes Single-Cell RNA Sequencing[a]

Characteristic	No History of Tuberculosis (n = 3)	History of Tuberculosis (n = 6)
Male sex, no. (%)	1 (33)	2 (33)
Age, mean, y	40.6 y	41.3 y
Recurrent tuberculosis, no. (%)	NA	3 (50)
Time since HIV diagnosis, median (IQR), y	6.78 (6.4–7.5)	6.4 (6.2–7.8)
Time since last completing tuberculosis treatment, median (IQR), y	NA	3.5 (0.9–4.2)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable; y, year.

[a]Participant-level demographics are detailed in Supplementary Table 2.

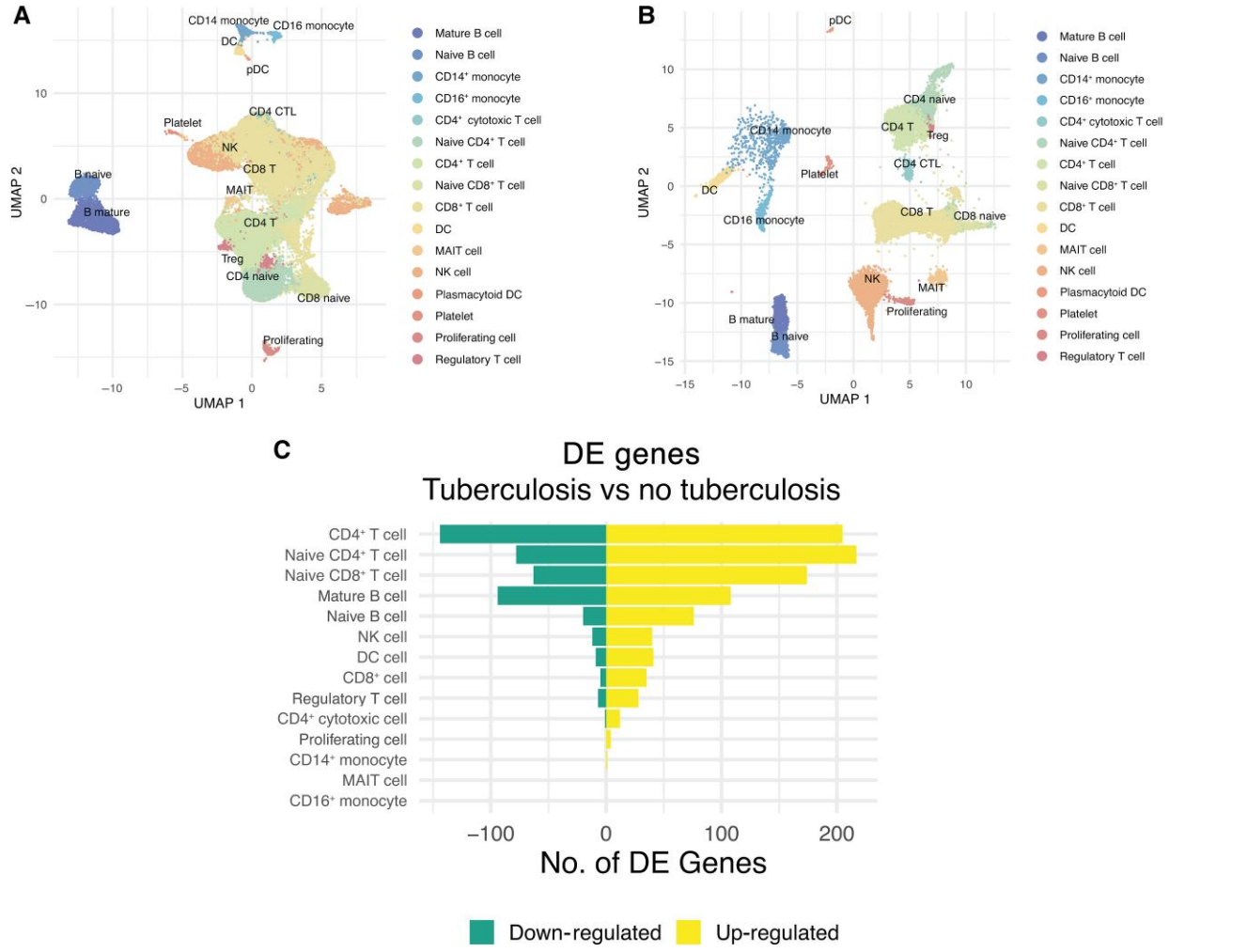

Figure 1. *A,* Gene expression data were used to cluster cells, using Seurat software with the Human Primary Cell Atlas reference. *B,* These data were also projected to the Azimuth peripheral blood mononuclear cell reference and clustered as expected. When tuberculosis history was considered as a binary variable (never tuberculosis vs ever tuberculosis), there were ≥40 differentially expressed genes (DE) with adjusted $P \leq .05$ and $|\log_2 \text{fold change}| > 0.25$ in each of the following populations: CD4$^+$, naive CD4$^+$, CD8$^+$, and naive CD8$^+$ T cells, naive and mature B cells, natural killer (NK) cells, and dendritic cells (DCs). *C,* In each cellular subset, there were genes with increased expression in the tuberculosis group (*right side of the graph*) and others with decreased expression (*left side of the graph*). Abbreviations: CTL, cytotoxic T lymphocytes; MAIT, mucosal-associated invariant T; pDC, plasmacytoid DC; Treg, regulatory T; UMAP, Uniform Manifold Approximation and Projection.

was considered as a binary variable (never tuberculosis vs ever tuberculosis), there were ≥40 differentially expressed genes with $P_{adj} \leq .05$ and $|\log_2 FC| \geq 0.25$ in each of the following populations: CD4$^+$, naive CD4$^+$, CD8$^+$, and naive CD8$^+$ T cells, naive and mature B cells, natural killer (NK) cells, and DCs (Figure 1*C*).

Differential Gene Expression and Pathway Analysis

In CD4$^+$ T cells, the significantly differentially expressed genes delineated tuberculosis and no-tuberculosis groups in the principle components analysis (Figure 2*A*). There were 349 differentially expressed genes with $P_{adj} \leq .05$ and $|\log_2 FC| \geq 0.25$ in CD4$^+$ T cells of tuberculosis versus no-tuberculosis groups (Figure 2*B*). The top GO biological pathways among genes upregulated in CD4+ T cells in the TB group included "response to topologically incorrect protein; and ;response to unfolded protein; (Figure 2*C*). The top GSEA hallmark pathway (normalized enrichment score [NES], 2.97; $P = 6.1 \times 10^{-23}$) consisted of "genes regulated by NF-κB in response to TNF [tumor necrosis factor]" [41].

There were 40 differentially expressed genes in CD8$^+$ T cells for people with a history of tuberculosis but 237 in the naive CD8$^+$ T-cell population (Supplementary Figure 2*A* and 2*B*). The top GSEA hallmark pathway for naive CD8$^+$ was also genes regulated by NF-κB in response to TNF (NES, 3.37; $P = 5.9 \times 10^{-37}$). The top biological function GO pathway was "integrated stress response signaling; followed by "intrinsic apoptotic signaling pathway; (Supplementary Figure 2*C*).

Naive B cells from people living with HIV with a history of tuberculosis had 96 differentially expressed genes (Supplementary Figure 3*A* and 3*B*). The top GSEA hallmark pathway was TNF signaling (NES, 3.24; $P = 6 \times 10^{-31}$), and the top GO biological

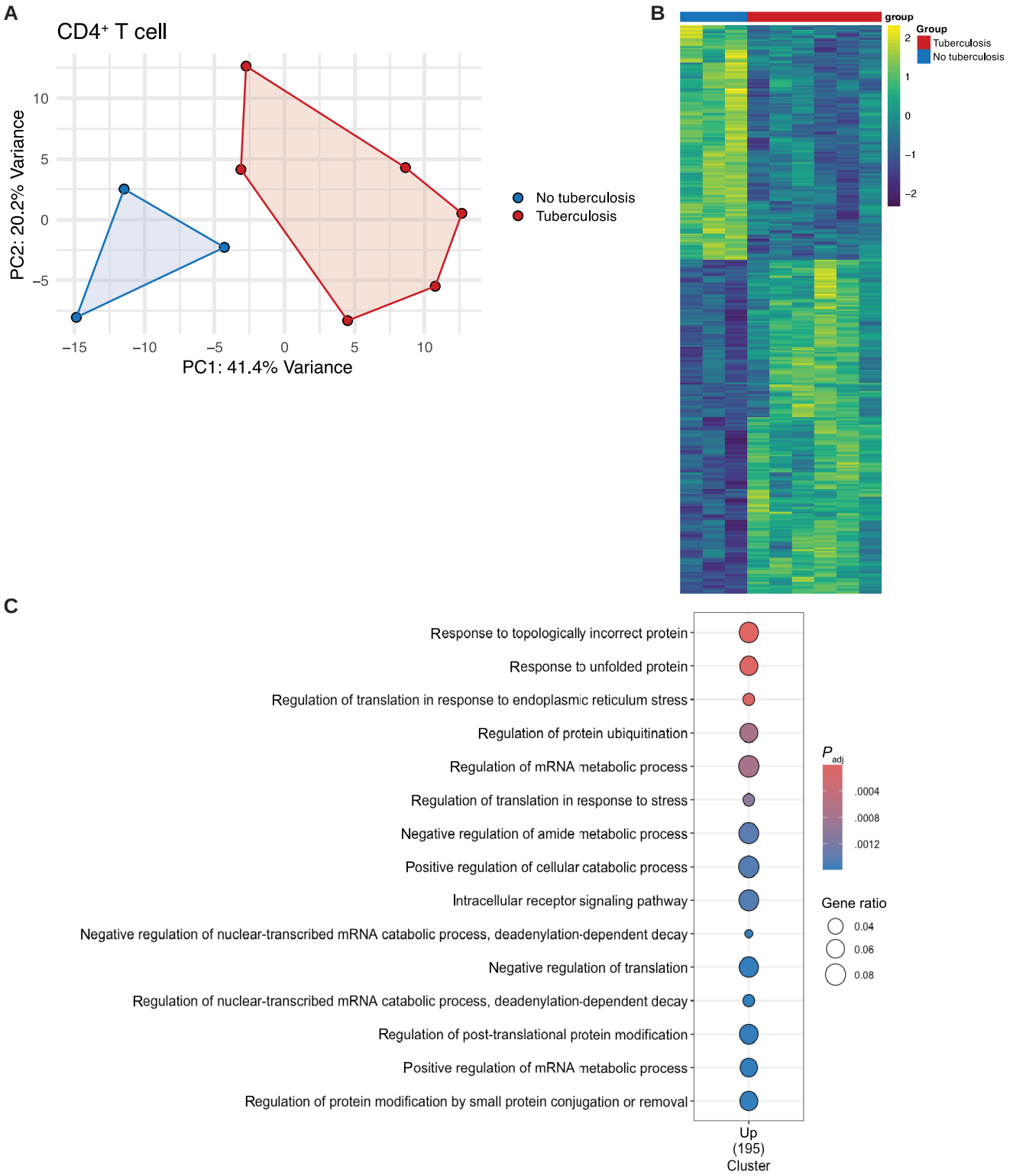

Figure 2. *A,* CD4$^+$ T cells cluster by tuberculosis status in principal components (PC) analysis of gene expression data. *B,* As shown in the heat map, there were 349 differentially expressed genes with adjusted P (P_{adj}) $\leq .05$ and $|\log_2$ fold change$| > 0.25$ in CD4$^+$ T cells of tuberculosis versus no-tuberculosis groups. *C,* The top Gene Ontology pathways among genes upregulated in CD4$^+$ T cells in the TB group were "response to topologically incorrect protein" and "response to unfolded protein." There were 195 genes upregulated in the TB group which overlapped with the genes used for the enrichment analysis. Abbreviation: mRNA, messenger RNA.

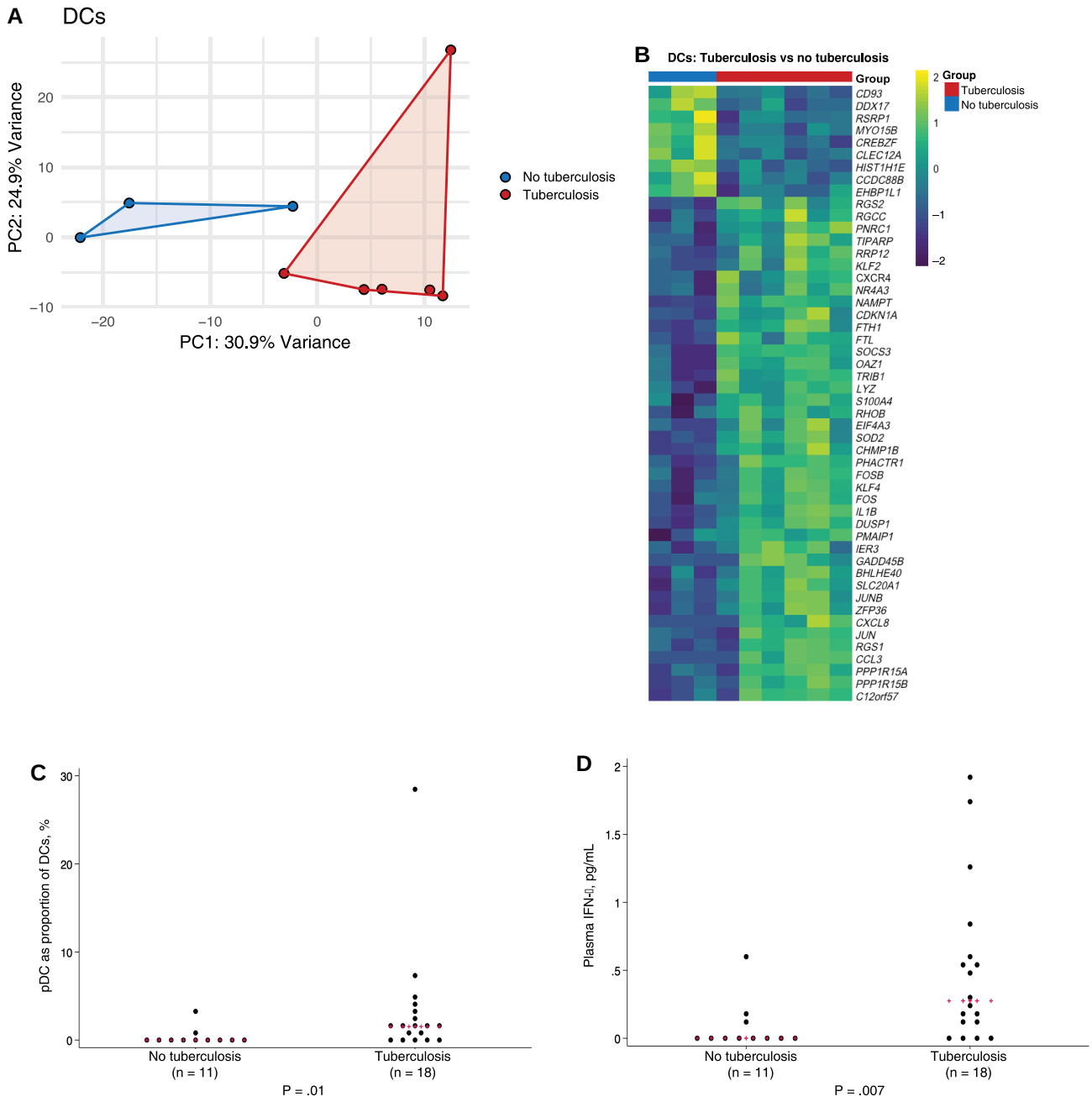

Figure 3. *A,* Dendritic cells (DCs) clustered by tuberculosis status in the principal components (PC) analysis of gene expression data. *B,* As shown in the heat map, there were 68 differentially expressed genes at adjusted $P \leq .05$ and $|\log_2 \text{fold change}| > 0.25$ in DCs from the tuberculosis versus no-tuberculosis groups. Each row shows the relative expression of each differentially expressed gene with the National Center for Biotechnology Information gene identifier shown to the right of each row. C, The tuberculosis group had a greater percentage of plasmacytoid DCs (pDCs) than the no-tuberculosis group when assessed with flow cytometry (*C*). *D,* pDCs are a key producer of interferon (IFN) α, and there were higher plasma levels of IFN-α in the tuberculosis group. Plus signs (+++) indicate medians. *P* values in *C* and *D* were generated using the Mann-Whitney *U* test for nonparametric data.

process pathways included ;negative regulation of protein phosphorylation; and ;phosphate/phosphorus metabolic processes; (Supplementary Figure 3*C*). There were 202 differentially expressed genes in mature B cells (Supplementary Figure 4*A* and 4*B*), with the same top GSEA hallmark pathway (NES, 2.56; $P = 3 \times 10^{-12}$) and top biological process GO terms related to translation regulation (Supplementary Figure 4*C*). Gene expression patterns clustered the tuberculosis versus no-tuberculosis groups in principal components analysis for both naive and mature B cells (Supplementary Figures 3*A* and 4*A*).

DCs from people living with HIV with history of tuberculosis had 68 differentially expressed genes (Figure 3*A* and 3*B*). Overrepresented GO pathways included responses to hydrogen peroxide, oxidative stress, and reactive oxygen species (Supplementary Figure 5*A*). The top GSEA hallmark pathway for DC was also TNF signaling via NF-κB. Using the published transcriptional markers of the DC clusters described by Villani et al [26] with our data, most DCs were type 2 (DC2). There was also a small cluster enriched for DC6, which includes the pDCs (Supplementary Figure 5*B*). We were unable to perform differential gene expression and pathway analysis comparing the tuberculosis versus no-tuberculosis groups in the pDC-containing DC6 subset because of the small number of pDCs in the no-tuberculosis group.

The TNF signaling via NF-κB pathway was the most significantly overrepresented hallmark pathway in all immune cell types, with ≥40 genes differentially expressed between the tuberculosis and no-tuberculosis groups. There were more cells with enriched gene expression in the TNF signaling via NF-κB pathway in the tuberculosis group (Figure 4*A*). The violin plot for each immune cell type is shown in Figure 4*B*. In the expanded cohort described below, the plasma concentration of TNF was significantly higher in the tuberculosis group (median [interquartile range], 3.3 [1.7–6] vs 0.76 [0–2.1] pg/mL; $P = .002$) (Figure 4*C*).

We subtyped DCs in a larger sample size of people living with HIV with or without a history of tuberculosis using flow cytometry. The final analyzed cohort (n = 29) consisted of 11 people living with HIV with no history of tuberculosis and 18 with a history of tuberculosis (Table 2), including the 9 who had FLEX CITE-Seq. While there were no significant differences in proportions of DC1 and DC2 (Supplementary Figure 5*C* and 5*D*) within DCs, people with a history of tuberculosis had more pDCs as a percentage of DCs ($P = .01$) (Figure 3*C*), including when the outlier in the tuberculosis group was excluded ($P = 0.01$). With tobit regression and including sex as a covariate, the larger number of pDCs in the tuberculosis group remained statistically significant ($P = .04$). There was a higher concentration of IFN-α in plasma from the tuberculosis group ($P = .007$) (Figure 3*D*). Because of potential left censoring of IFN-α concentrations, we applied tobit regression with and without sex as a covariate (both $P = .01$). The IFN-α concentration was not correlated with the pDC percentage ($R = -0.05$; $P = .79$) (Supplementary Figure 5*E*).

For the secondary analysis, 4 cytokines in the Luminex panel were excluded because of >50% with levels below the lower limit of calculation. After Bonferroni correction for the remaining analyzable cytokines, our cutoff value for statistical significance was $P_{adj} \leq .004$ (0.05/13). The cytokines that remained significantly elevated in people living with HIV with a history of tuberculosis after Bonferroni correction were interleukin 2, 6, 12p70, and 15, and TNF (Table 3, Supplementary Figure 6*A*–6*D*, and Figure 4*C*). Each of these cytokines remained statistically significant ($P < .01$) using tobit regression with tuberculosis as the independent variable, indicating that the differences were not confounded by left censoring of low cytokine values.

DISCUSSION

People living with HIV with a history of tuberculosis have worse outcomes than those who have never had active tuberculosis. We tested our hypothesis that there was an immunologic profile associated with tuberculosis history using PBMC scRNA-Seq, DC phenotyping, and plasma cytokine quantitation for people living with HIV with or without a history of tuberculosis. Immune pathways important for tuberculosis control were enriched in the differentially expressed genes in DCs from people living with HIV with versus without a history of tuberculosis, so we then quantitated the proportions of DC subtypes in circulating PBMCs. We found a significantly greater proportion of pDCs in people with a history of tuberculosis. pDCs are a minority of circulating PBMCs (0.2%–0.5%) [42] but produce up to 1000-fold higher IFN-α compared with other cells [43]. Thus, small differences in pDC quantity, as we observed, can impart large differences in inflammation.

As pDCs are involved in initial control of HIV as well as activation of provirus-containing $CD4^+$ T cells [19], they may contribute to higher levels of $CD4^+$ T cells containing intact HIV provirus, as previously reported in people with history of tuberculosis [19]. One scRNA-Seq study of people living with HIV with tuberculosis before treatment showed a distinct pDC population in the UMAP, but pDCs as a proportion of total PBMCs did not differ from a no-TB group [14]. Rambaran et al [44] reported a lower frequency of pDCs in people living with HIV with tuberculosis compared with healthy controls, but this was before treatment, and the comparison group was healthy community controls. In scRNA-Seq of DCs from 4 men living with HIV, there were 4 pDC subsets: pDC1, pDC2, a cytotoxic-like pDC, and an exhausted pDC [45]. It was predicted that pDC2 would produce IFN-α [45]. Our study did not have sufficient numbers of pDCs to differentiate them into the 4 separate pDC subsets, but this is an area for further investigation.

IFN-α and other type I IFNs promote host-protective responses during early *Mtb* infection but promote *Mtb* replication and destructive human pathologic immune responses later in infection [46]. IFN-α inhibits the establishment of HIV latency in $CD4^+$ T lymphocytes but also reverses latency once established [47]. In people with pulmonary tuberculosis without HIV coinfection, IFN-α expression may remain elevated at the end of 24 weeks of tuberculosis treatment [48, 49], but studies do not generally extend into the posttreatment period. Our findings suggest that elevated IFN-α levels persist even after effective treatment of pulmonary tuberculosis.

In addition to more pDCs and IFN-α, we found that people living with HIV with history of tuberculosis had increased

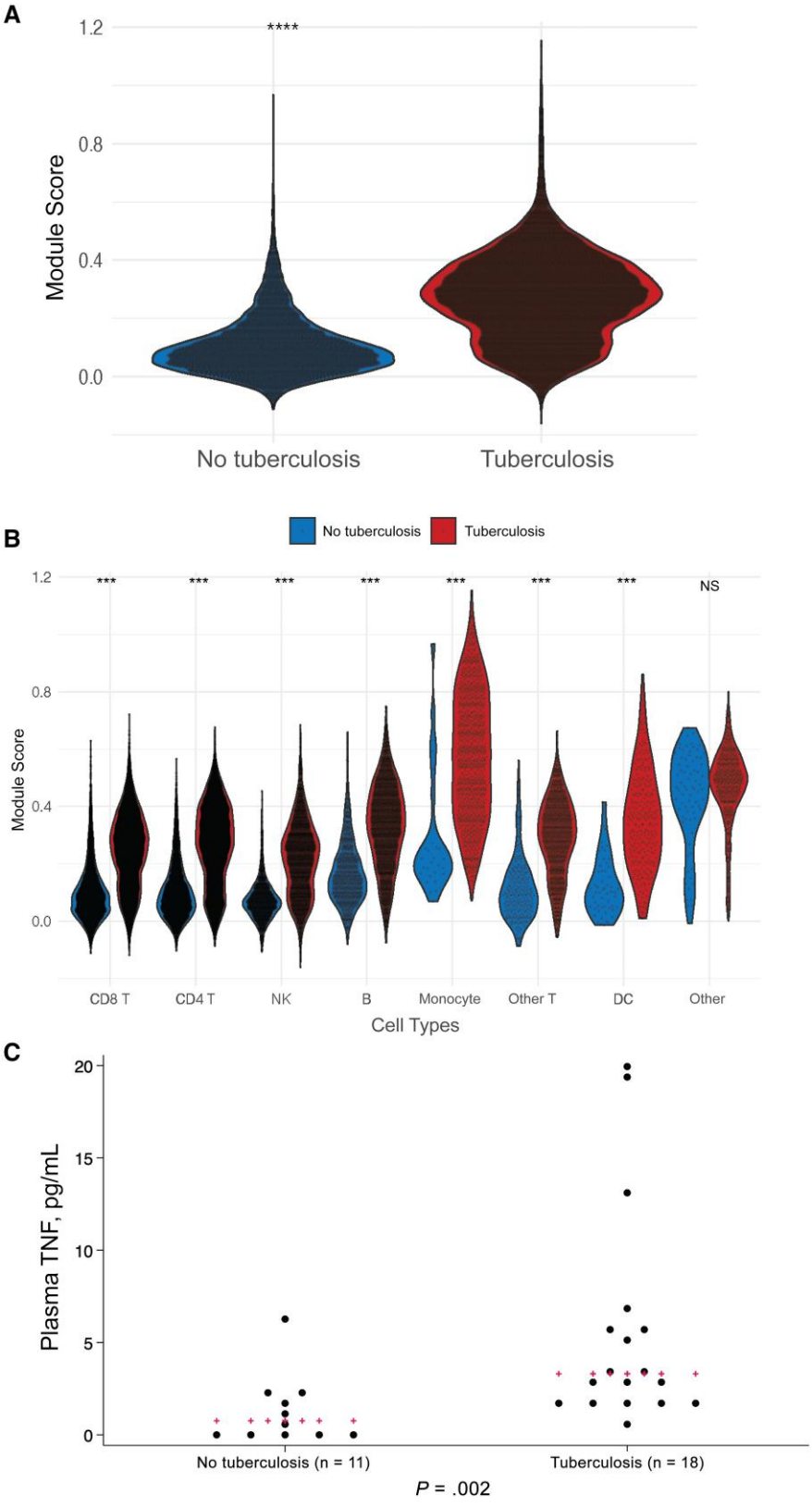

Figure 4. The top hallmark pathway for all cell types with ≥40 genes differentially expressed between the tuberculosis and no-tuberculosis groups was tumor necrosis factor (TNF) signaling via the NF-κB gene. *A,* Violin plot shows the overrepresentation of this pathway in the tuberculosis (*red*) compared with the no-tuberculosis (*blue*) group. *B,* This was also true when comparing expression of genes in this pathway between tuberculosis (*red*) and no-tuberculosis (*blue*) groups within the immune cell subsets, except for the "other" cells. Abbreviations: DC, dendritic cell; NK, natural killer; NS, not significant. ***$P < .001$ (Wilcoxon test). *C,* As a correlate of the importance of this pathway, we found increased plasma TNF levels in the tuberculosis group compared with the no-tuberculosis group. Plus signs (+++) indicate medians.

Table 2. Characteristics of Participants With Dendritic Cell Subtyping and Cytokine Assessment

Characteristic	No History of Tuberculosis (n = 11)	History of Tuberculosis (n = 18)
Male sex, no. (%)	5 (45)	8 (44)
Age, mean age (95% SD), y	42 (11.9)	45 (9.1)
Recurrent tuberculosis, no. (%)	NA	9 (50)
Time since HIV diagnosis, median in years (IQR), y	6.36 (4.7–7.9)	7.0 (5.9–9.7)
Time since last completing tuberculosis treatment, median (IQR), y	NA	3.0 (1.5–4.2)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable; y, year.

Table 3. Plasma Cytokine Levels in People With HIV With or Without a History of Tuberculosis

Cytokine	Cytokine Level, Median (IQR), pg/mL		*P* Value[a]
	No History of Tuberculosis (n = 11)	History of Tuberculosis (n = 18)	
IFN-α	0 (0–0.1)	0.28 (0.1–0.61)	.007
IL-2	1.91 (0–6.24)	7.7 (5.19–15.87)	.002
IL-6	3.1 (0–4.59)	8.3 (6.1–26.1)	<.001
IL-12p70	0 (0–0.8)	2.75 (1.24–6.38)	<.001
IL-15	1.22 (0–3.81)	4.1 (3.2–9.4)	.003
TNF	0.76 (0–2.1)	3.3 (1.7–6)	.002

Abbreviations: IFN, interferon; IL-2, IL-6, IL-12p70, and IL-15, interleukin 2, 6, 12p70, and 15; TNF, tumor necrosis factor.

[a] *P* values based on Mann-Whitney *U* test for nonparametric data.

expression of genes regulated by NF-κB in response to TNF in nearly all immune cellular subsets examined. Higher plasma TNF levels in those with a history of tuberculosis supports the significance of this pathway in that population. In addition to higher TNF levels, there were higher levels of IFN-α and interleukin 2, 12p70, 6, and 15 in plasma, all proinflammatory cytokines associated with immune activation.

Strengths of the study include leveraging PBMCs collected in clinical settings from people who have had HIV infection for years to see the long-term impact of well-controlled HIV on the immune milieu. This is critical since cell lines infected in the laboratory do not recapitulate the biology of natural infection [50]. Although our sample size was small, we had ≥80% power to detect statistically significant differences in gene expression with FLEX CITE-Seq. Our study design was strengthened by matching tuberculosis case patients and no-tuberculosis controls by sex, age, and time since HIV diagnosis. In addition, all participants were recruited from the same geographic area and had similar socioeconomic circumstances, which helps control for the impact of unquantifiable variables on our findings.

Limitations of the study include the single time point of immune phenotyping, which was years after tuberculosis treatment, so we could not assess the temporality of the immune changes. Another limitation is the size of the cohort who received CITE-Seq. However, even with a small sample size, we had 80% power to detect differential gene expression in $CD4^+$ T cells in PBMCs. In addition, to confirm the findings we saw in immune cells, we designed a more expansive study of DC phenotypes and circulating cytokines powered for differences in pDCs seen in the original cohort of 9 people.

We were not able to include nadir $CD4^+$ T-cell count or peak HIV viral load as a covariate in these analyses because our participants were recruited from a clinical setting with significant obstacles to frequent $CD4^+$ T-cell counts and viral load measurements. We include the *measured* nadir $CD4^+$ T-cell count available but did not include these counts as covariates because we know that they are not actual nadirs based on the timing of the blood collection relative to HIV diagnosis and the start of antiretroviral therapy start. Despite this limitation, we believe it is imperative to include people in translational HIV research who do not have access to CD4 monitoring because of the resources available where they live. This is especially important as we understand more about the impact of coinfections such as *M. tuberculosis*, which may be endemic in these areas, for people living with HIV.

These findings of distinct immunologic profiles among people living with HIV with a history of treated tuberculosis are important for understanding the long-term sequelae of tuberculosis coinfection. We propose additional studies to determine why pDCs remain elevated in people living with HIV after tuberculosis cure, deep phenotyping of DCs to determine their contribution to the elevations in proinflammatory cytokines during tuberculosis and after tuberculosis cure, and studies to determine the role of continued environmental tuberculosis antigen exposure on systemic inflammation. We will investigate whether there is an expansion of *Mtb*-specific immune cells by phenotyping *Mtb*-stimulated PBMCs and characterizing the T cell receptor and B cell receptor repertoire, comparing people with versus without a history of pulmonary tuberculosis. Our findings are an additional piece of the puzzle of why people living with HIV with a history of tuberculosis have worse clinical outcomes even after tuberculosis cure.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We gratefully acknowledge all of our study participants and the patient care, clinical research, and laboratory teams at GHESKIO Centers in Port au Prince Haiti. We especially thank the GHESKIO laboratory team members who process blood for PBMC isolation and storage, without whom this study would not have been possible. We acknowledge productive scientific discussions with Selin Somersan, MD, and Sabine Ehrt, PhD. We also thank the Epigenomics Core Laboratory at Weill Cornell Medicine for implementation of the Chromium Single Cell Gene Expression FLEX for fixed cells.

Author contributions. Conceptualization: D. W. F., J. W. P., and K. M. D. Methodology: M. W., P. Z., A. Apollon, A. Alonso, D. B., and K. M. D. Investigation: M. W., Y. J., and K. M. D. Resources: Y. J., D. B., D. W. F., and J. W. P. Funding acquisition: D. W. F. and K. M. D. Project administration: Y. J., A. Apollon, and A. Alonso. Software and data curation: P. Z. Formal analysis and visualization: P. Z. and K. M. D. Supervision: J. W. P. and K. M. D. Writing—original draft: M. W., D. W. F., and K. M. D. Writing—review: M. W., P. Z., A. Alonso, D. B., and K. M. D.

Financial support. This work was supported by the National Institute of Allergy and Infectious Disease (grant P30-AI168433, grant K23-AI131913 to K. M. D., and R01-AI176943 to K. M. D.), the National Center for Advancing Translational Sciences (grant UL1-TR002385), and the Doris Duke Charitable Foundation (Clinical Scientist Development Award to K. M. D.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Joseph Y, Yao Z, Dua A, et al. Long-term mortality after tuberculosis treatment among persons living with HIV in Haiti. J Int AIDS Soc **2021**; 24:e25721.
2. Olson A, Ragan EJ, Nakiyingi L, et al. Brief report: pulmonary tuberculosis is associated with persistent systemic inflammation and decreased HIV-1 reservoir markers in coinfected Ugandans. J Acquir Immune Defic Syndr **2018**; 79:407–11.
3. Kraef C, Tusch E, Singh S, et al. All-cause and AIDS-related mortality among people with HIV across Europe from 2001 to 2020: impact of antiretroviral therapy, tuberculosis and regional differences in a multicentre cohort study. Lancet Reg Health Eur **2024**; 44:100989.
4. Whalen CC, Nsubuga P, Okwera A, et al. Impact of pulmonary tuberculosis on survival of HIV-infected adults: a prospective epidemiologic study in Uganda. AIDS **2000**; 14: 1219–28.
5. Bisson GP, Zetola N, Collman RG. Persistent high mortality in advanced HIV/TB despite appropriate antiretroviral and antitubercular therapy: an emerging challenge. Curr HIV/AIDS Rep **2015**; 12:107–16.
6. Lumu I, Musaazi J, Semeere A, Handel I, Castelnuovo B. Survival and predictors of mortality after completion of TB treatment among people living with HIV: a 5-year analytical cohort. BMC Infect Dis **2023**; 23:238.
7. Zaongo SD, Harypursat V, Chen Y. Single-cell sequencing facilitates elucidation of HIV immunopathogenesis: a review of current literature. Front Immunol **2022**; 13: 828860.
8. Kulkarni S, Endsley JJ, Lai Z, Bradley T, Sharan R. Single-cell transcriptomics of Mtb/HIV co-infection. Cells **2023**; 12:2295.
9. Cai Y, Dai Y, Wang Y, et al. Single-cell transcriptomics of blood reveals a natural killer cell subset depletion in tuberculosis. EBioMedicine **2020**; 53:102686.
10. Pan J, Zhang X, Xu J, Chang Z, Xin Z, Wang G. Landscape of exhausted T cells in tuberculosis revealed by single-cell sequencing. Microbiol Spectr **2023**; 11:e0283922.
11. Hillman H, Khan N, Singhania A, et al. Single-cell profiling reveals distinct subsets of CD14+ monocytes drive blood immune signatures of active tuberculosis. Front Immunol **2022**; 13:1087010.
12. Jiang J, Cao Z, Li B, et al. Disseminated tuberculosis is associated with impaired T cell immunity mediated by non-canonical NF-κB pathway. J Infect **2024**; 89:106231.
13. Wang Y, Sun Q, Zhang Y, et al. Systemic immune dysregulation in severe tuberculosis patients revealed by a single-cell transcriptome atlas. J Infect **2023**; 86:421–38.
14. Guo Q, Zhong Y, Wang Z, et al. Single-cell transcriptomic landscape identifies the expansion of peripheral blood monocytes as an indicator of HIV-1-TB co-infection. Cell Insight **2022**; 1:100005.
15. Kgoadi K, Bajpai P, Ibegbu CC, et al. Alveolar macrophages from persons with HIV mount impaired TNF signaling networks to M. tuberculosis infection. Nat Commun **2025**; 16:2397.
16. Heubeck AT, Phalen C, Kaul N, et al. CryoSCAPE: scalable immune profiling using cryopreserved whole blood for multi-omic single cell and functional assays. J Transl Med **2025**; 23:5.
17. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods **2017**; 14:865–8.
18. World Health Organization. Tuberculosis profile: Haiti. https://worldhealthorg.shinyapps.io/tb_profiles/?_inputs_&entity_type=%22country%22&iso2=%22HT%22&lan=%22EN%22. Accessed 9 October 2025.
19. Juste MAJ, Joseph Y, Lespinasse D, et al. People living with HIV have more intact HIV DNA in circulating CD4+ T

cells if they have history of pulmonary tuberculosis. Pathog Immun **2024**; 9:172–93.
20. Osorio D, Cai JJ. Systematic determination of the mitochondrial proportion in human and mice tissues for single-cell RNA-sequencing data quality control. Bioinformatics **2021**; 37:963–7.
21. Germain PL, Lun A, Garcia Meixide C, Macnair W, Robinson MD. Doublet identification in single-cell sequencing data using scDblFinder. F1000Res **2021**; 10:979.
22. Hao Y, Hao S, Andersen-Nissen E, et al. Integrated analysis of multimodal single-cell data. Cell **2021**; 184:3573–87.e29.
23. Aran D, Looney AP, Liu L, et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nat Immunol **2019**; 20:163–72.
24. Mabbott NA, Baillie JK, Brown H, Freeman TC, Hume DA. An expression atlas of human primary cells: inference of gene function from coexpression networks. BMC Genomics **2013**; 14:632.
25. Stuart T, Butler A, Hoffman P, et al. Comprehensive integration of single-cell data. Cell **2019**; 177:1888–902.e21.
26. Villani AC, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science **2017**; 356:eaah4573.
27. Hambleton S, Salem S, Bustamante J, et al. *IRF8* mutations and human dendritic-cell immunodeficiency. N Engl J Med **2011**; 365:127–38.
28. Kong XF, Martinez-Barricarte R, Kennedy J, et al. Disruption of an antimycobacterial circuit between dendritic and helper T cells in human SPPL2a deficiency. Nat Immunol **2018**; 19:973–85.
29. Schutz F, Hackstein H. Identification of novel dendritic cell subset markers in human blood. Biochem Biophys Res Commun **2014**; 443:453–7.
30. Heinig Lab. scPower: a statistical framework for design and power analysis of multi-sample single cell transcriptomics experiments. https://scpower.helmholtz-muenchen.de/. Accessed 5 August 2025.
31. Schmid KT, Hollbacher B, Cruceanu C, et al. scPower accelerates and optimizes the design of multi-sample single cell transcriptomic studies. Nat Commun **2021**; 12:6625.
32. Squair JW, Gautier M, Kathe C, et al. Confronting false discoveries in single-cell differential expression. Nat Commun **2021**; 12:5692.
33. Murphy AE, Skene NG. A balanced measure shows superior performance of pseudobulk methods in single-cell RNA-sequencing analysis. Nat Commun **2022**; 13:7851.
34. Skinnider M, Squair J. Libra: Differential expression utilizing biological replicates in single-cell data. R package version 1.0.0 ed. 2025. https://github.com/neurorestore/Libra.
35. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol **2014**; 15:550.
36. Aleksander SA, Balhoff J, Carbon S, et al; Gene Ontology Consortium. The Gene Ontology knowledgebase in 2023. Genetics **2023**; 224:iyad031.
37. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation (Camb) **2021**; 2:100141.
38. Korotkevich G, Sukhov V, Budin N, Shpak B, Artyomov MN, Sergushichev A. Fast gene set enrichment analysis. bioRxiv [Preprint: not peer reviewed]. 1 February 2021. Available from: https://www.biorxiv.org/content/10.1101/060012v3.].
39. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A **2005**; 102:15545–50.
40. Fitzgerald DW, Marotte C, Verdier RI, Johnson WD, Pape J, W J. Comprehension during informed consent in a less-developed country. Lancet **2002**; 360:1301–2.
41. Hayden MS, Ghosh S. Regulation of NF-κB by TNF family cytokines. Semin Immunol **2014**; 26:253–66.
42. Fitzgerald-Bocarsly P, Jacobs ES. Plasmacytoid dendritic cells in HIV infection: striking a delicate balance. J Leukoc Biol **2010**; 87:609–20.
43. Anes E, Azevedo-Pereira JM, Pires D. Role of type I interferons during *Mycobacterium tuberculosis* and HIV infections. Biomolecules **2024**; 14:848.
44. Rambaran S, Maseko TG, Lewis L, et al. Blood monocyte and dendritic cell profiles among people living with HIV with *Mycobacterium tuberculosis* co-infection. BMC Immunol **2023**; 24:21.
45. Cham LB, Gunst JD, Schleimann MH, et al. Single cell analysis reveals a subset of cytotoxic-like plasmacytoid dendritic cells in people with HIV-1. iScience **2023**; 26:107628.
46. Moreira-Teixeira L, Mayer-Barber K, Sher A, O'Garra A. Type I interferons in tuberculosis: foe and occasionally friend. J Exp Med **2018**; 215:1273–85.
47. Van der Sluis RM, Zerbato JM, Rhodes JW, et al. Diverse effects of interferon alpha on the establishment and reversal of HIV latency. PLoS Pathog **2020**; 16:e1008151.
48. Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, et al. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: a prospective cohort study. Cytokine **2019**; 123:154759.
49. Lichtner M, Rossi R, Mengoni F, et al. Circulating dendritic cells and interferon-α production in patients with tuberculosis: correlation with clinical outcome and treatment response. Clin Exp Immunol **2006**; 143:329–37.
50. Chingwaru W, Glashoff RH, Vidmar J, Kapewangolo P, Sampson SL. Mammalian cell cultures as models for *Mycobacterium tuberculosis*-human immunodeficiency virus (HIV) interaction studies: a review. Asian Pac J Trop Med **2016**; 9:832–8.