Activation Profile of *Mycobacterium tuberculosis*–Specific CD4$^+$ T Cells Reflects Disease Activity Irrespective of HIV Status

To the Editor:

The diagnosis of pulmonary tuberculosis in HIV-infected individuals is particularly challenging as HIV-induced alterations of the immune system lead to reduced cavitation, limiting the sensitivity of sputum-based assays (1). Thus, alternate markers are needed to distinguish between latent tuberculosis infection (LTBI) and active tuberculosis (aTB) in this high-risk group. Several attributes of *Mycobacterium tuberculosis* (Mtb)-specific CD4$^+$ T cells have been shown to efficiently delineate LTBI and aTB in HIV-uninfected individuals, including their polyfunctional or memory profiles (2–4). Moreover, Adekambi and colleagues recently demonstrated that the activation profile of Mtb-specific CD4$^+$ T cells accurately discriminates between LTBI and aTB (5). As chronic HIV infection is characterized by persistent systemic immune activation (6), it is plausible that these blood-based markers may not be relevant for HIV-infected individuals.

We, therefore, compared the potential of the activation and polyfunctional profiles of Mtb-specific CD4$^+$ T cells to distinguish between LTBI and aTB in HIV-uninfected and HIV-infected individuals. We analyzed 76 participants divided in four groups according to their TB and HIV status: LTBI/HIV$^-$ (n = 17; median age, 22 yr; 47% female), aTB/HIV$^-$ (n = 17; median age, 27 yr; 29% female), LTBI/HIV$^+$ (n = 21; median age, 29 yr; 67% female; median CD4 count, 316 cells/mm^3; interquartile range [IQR], 231–543 cells/mm^3), and aTB/HIV$^+$ (n = 21; median age, 35 yr; 57% female; CD4 count, 250 cells/mm^3; IQR, 155–295 cells/mm^3). LTBI was defined as tuberculin skin test positive, IFN-γ release assay positive, sputum culture negative, and normal chest X-ray. aTB was diagnosed on the basis of symptoms suggestive of tuberculosis and Mtb-positive smear and/or sputum culture, as previously described (7). All HIV-infected participants were antiretroviral therapy–naive. The University of Cape Town ethics committee approved the study, and written consent was obtained from participants. Cryopreserved peripheral blood mononuclear cells were stimulated for 16 hours with early secretory antigenic target-6 (ESAT-6)/culture filtrate protein-10 (CFP-10) peptide pool, and intracellular staining, using a live/dead marker and antibodies toward CD3, CD4, CD8, human leukocyte antigen-DR (HLA-DR), Ki67, CD38, IFN-γ, tumor necrosis factor (TNF)-α, and IL-2, was performed. Positive ESAT-6/CFP-10 responses (defined as twice the background) were detectable in 16 subjects in the LTBI/HIV$^-$ and LTBI/HIV$^+$ groups, and in 15 and 18 individuals in the aTB/HIV$^-$ and aTB/HIV$^+$ groups, respectively. No significant differences were observed in the overall magnitude of IFN-γ$^+$ responses between the four groups (data not shown).

We first compared the activation profile of IFN-γ$^+$ Mtb-specific CD4$^+$ T cells between the four groups (Figure 1A). As previously shown (5), in HIV-uninfected persons, HLA-DR, Ki67, and CD38 expression on IFN-γ$^+$ Mtb-specific CD4$^+$ T cells was significantly higher in aTB participants than in those with LTBI (Figure 1B). Interestingly, although HLA-DR expression on Mtb-specific CD4$^+$ T cells in the LTBI/HIV$^+$ group (median, 41.7%; IQR, 25.7–54.6%) was significantly higher than in the LTBI/HIV$^-$ group (median, 13.7%; IQR, 8.9–27.5%), HLA-DR expression on these cells was significantly further increased in HIV-infected individuals with aTB (median, 84%; IQR, 73.7–87.9%) (Figure 1B). Additional analyses showed that in LTBI/HIV$^+$ individuals, HLA-DR expression on Mtb-specific CD4$^+$ T cells mirrors HLA-DR expression in the whole CD4 compartment ($P = 0.02$; $r = 0.56$), but this association was not apparent in aTB/HIV$^+$ individuals (data not shown). Unlike HLA-DR, Ki67 and CD38 expression levels were comparable between HIV-uninfected and HIV-infected individuals with LTBI. In HIV-infected persons with aTB, Ki67 expression on IFN-γ$^+$ Mtb-specific CD4$^+$ T cells was significantly higher ($P < 0.0001$) than in LTBI, whereas the up-regulation of CD38 was more modest between these two groups ($P = 0.03$). Of note, in the aTB/HIV$^+$ group, the expression of CD38 was significantly higher in individuals with a positive smear when compared with smear-negative participants ($P = 0.01$; data not shown), suggesting that CD38 expression could reflect bacterial load. To assess the accuracy of these markers to discriminate between LTBI and aTB status, receiver operating characteristic curves and crossover plots were performed. Figure 1C shows the data for HLA-DR; area under the curve (AUC) and *P* values reflect that HLA-DR expression on IFN-γ$^+$ Mtb-specific CD4$^+$ T cells distinguishes LTBI and aTB in both the HIV$^-$ and HIV$^+$ groups (AUC = 0.98 [$P < .0001$]; and AUC = 0.9 [$P < 0.0001$], respectively). However, the optimum cutoff values discriminating LTBI from aTB were distinct for HIV-uninfected (40%) and HIV-infected (70%) individuals. In our experimental setting, the expression of Ki67 and CD38 was less robust to differentiate TB status in HIV-uninfected (AUC = 0.896 [$P = 0.00017$], cutoff = 1.4%; AUC = 0.858 [$P = 0.0007$], cutoff = 4%, respectively) and HIV-infected (AUC = 0.89 [$P = 0.0002$], cutoff = 2.4%; AUC = 0.72 [$P = 0.026$], cutoff = 5%, respectively) individuals (data not shown). Our data were comparable to those of Adekambi and colleagues (5) despite disparity in the cutoff value for these markers, which could be explained by flow-cytometry technical differences.

The polyfunctional profile of Mtb-specific CD4$^+$ T cells has also been shown to discriminate between LTBI and aTB in HIV-uninfected individuals (2, 3), but conflicting data exist for HIV-infected persons (8–10). Thus, we compared the profile of ESAT-6/CFP-10-specific CD4$^+$ T cells on the basis of their capacity to secrete IFN-γ, TNF-α, and/or IL-2, between the four groups (Figure 2A). HIV-uninfected individuals with LTBI were

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Author Contributions: C.R. and K.A.W. designed the study; K.A.W. performed the experiments; C.R. and K.A.W. analyzed the data; T.O., H.P.G., and R.G. contributed to patient recruitment and diagnosis and sample collection and storage; C.R., K.A.W., and R.J.W. drafted the manuscript; all authors read, critically revised, and approved the final manuscript.

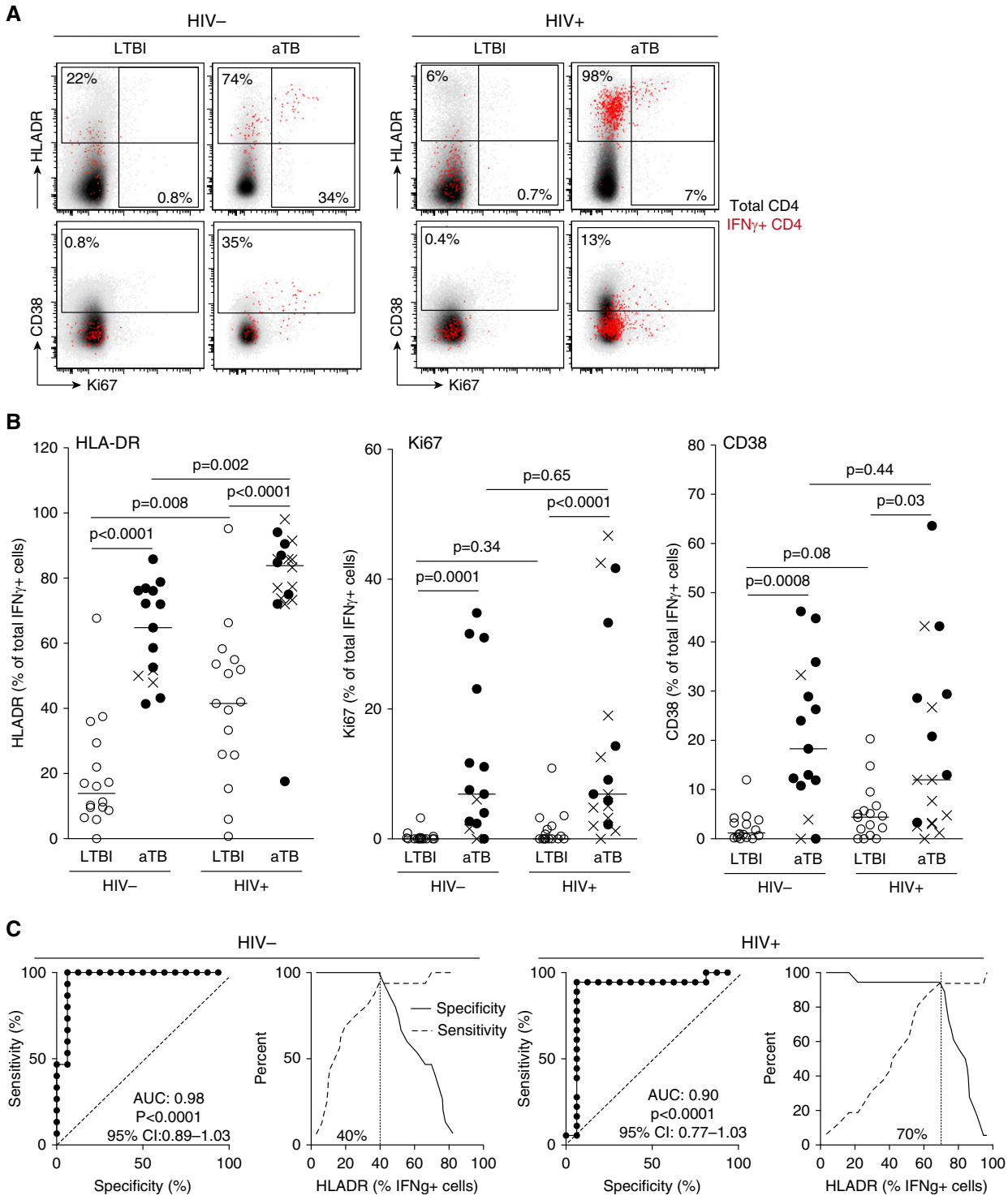

Figure 1. Comparison of the activation profile of IFN-γ^+ early secretory antigenic target-6/culture filtrate protein-10–specific $CD4^+$ T cells between HIV-uninfected and HIV-infected individuals with latent tuberculosis infection (LTBI) or active tuberculosis (aTB). (*A*) Representative overlay plots of human leukocyte antigen-DR (HLA-DR), CD38, and Ki-67 expression in total $CD4^+$ T cells (*gray*) and IFN-γ^+ *Mycobacterium tuberculosis* (Mtb)-specific $CD4^+$ T cells (*red*). (*B*) Expression of HLA-DR, Ki67, and CD38 on IFN-γ^+ Mtb-specific $CD4^+$ T cells in LTBI/HIV^- (n = 16), aTB/HIV^- (n = 15), LTBI /HIV^+ (n = 16), and aTB/HIV^+ (n = 18) participants. *Open circles* depict LTBI individuals, *solid circles* represent smear-positive patients with aTB, and *crosses* correspond to smear-negative and culture-positive individuals with aTB. *Horizontal lines* indicate the median. Statistical comparisons were performed using a nonparametric Mann-Whitney *U* test. (*C*) Receiver operating characteristic curves and specificity/sensitivity crossover plots for HLA-DR expression level in IFN-γ^+ Mtb-specific $CD4^+$ T cells to discriminate between LTBI or aTB in HIV-uninfected and HIV-infected individuals. The area under the curve (AUC), *P* value, and confidence intervals (CIs) are shown. The *diagonal dashed line* depicts an AUC of 0.5, representing a random test. The *dashed vertical line* on the crossover plots represents the optimal threshold to distinguish LTBI and aTB individuals.

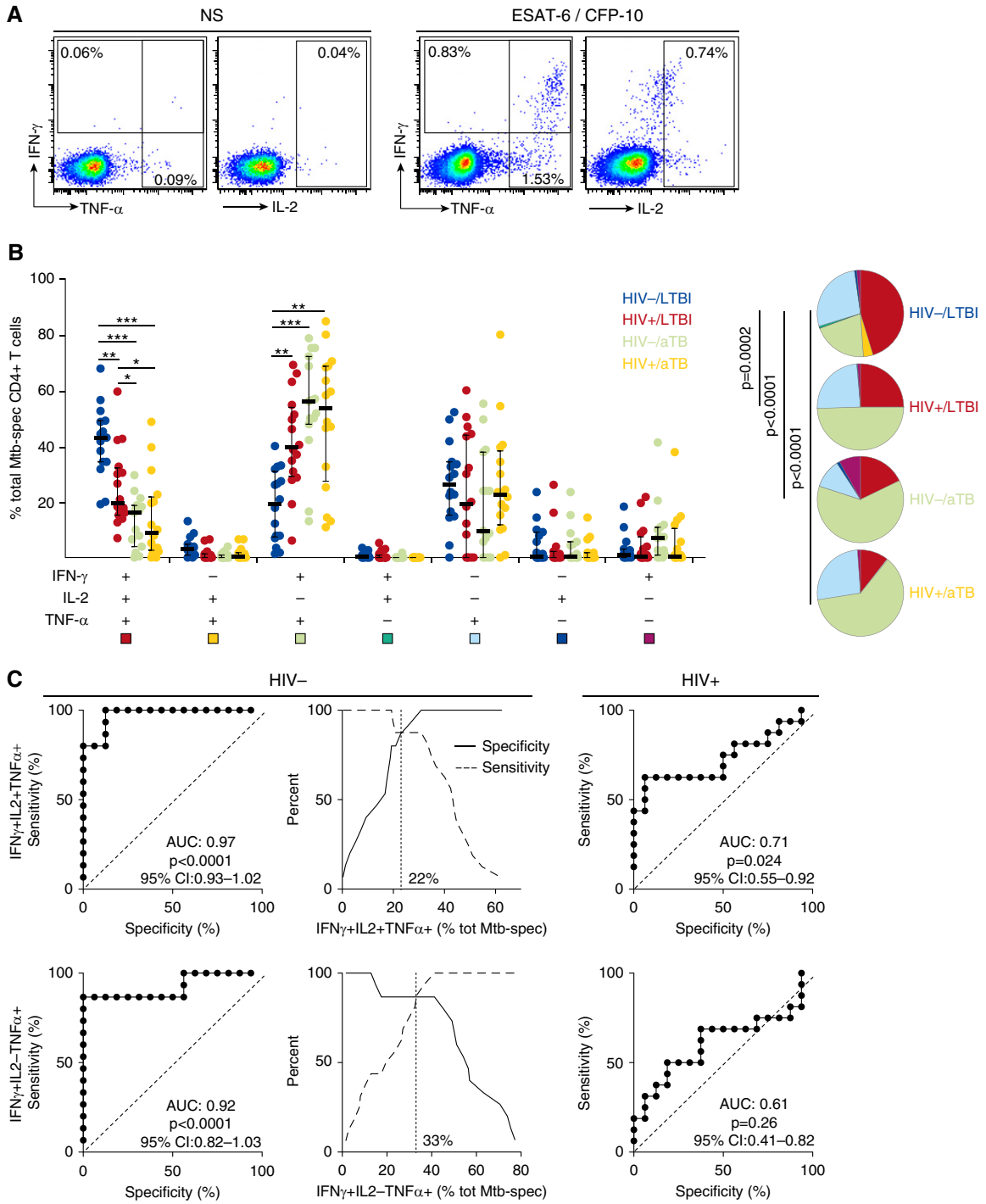

Figure 2. Comparison of the polyfunctional profile of early secretory antigenic target-6 (ESAT-6)/culture filtrate protein-10 (CFP-10)–specific $CD4^+$ T cells between HIV-uninfected and HIV-infected individuals with latent tuberculosis infection (LTBI) or active tuberculosis (aTB). (*A*) Representative dot plots of IFN-γ, tumor necrosis factor (TNF)-α, and IL-2 production in response to ESAT-6/CFP-10 peptide pool in one LTBI/HIV^- individual. NS = no stimulation. *Numbers* represent the frequencies of cytokine-producing cells expressed as a percentage of the total $CD4^+$ T-cell population. (*B*) Proportion of *Mycobacterium tuberculosis* (Mtb)-specific $CD4^+$ T cells producing any possible combinations of IFN-γ, TNF-α, or IL-2. *Horizontal bars* represent the median values and interquartile range. Statistical analysis was performed using Mann-Whitney test; significant differences are indicated by asterisks (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). Each *slice of the pie* corresponds to a distinct combination of cytokine. A key to colors used in the pie charts is shown at the bottom of the graph. (*C*) Receiver operating characteristic curves and specificity/sensitivity crossover plots for the proportion of IFN-γ^+ IL-2^+ TNF-α^+ (*top*), and IFN-γ^+ IL-2^-TNF-α^+ (*bottom*) Mtb-specific $CD4^+$ T cells to discriminate between LTBI or aTB in HIV-uninfected and HIV-infected individuals. The *diagonal dashed line* depicts an area under the curve (AUC) of 0.5, representing a random test. The *dashed vertical line* on the crossover plots represents the optimal threshold to distinguish LTBI and aTB individuals. CI = confidence interval.

characterized by a predominant proportion of IFN-γ^+IL-2^+TNF-α^+ cells (median, 44%; IQR, 35–49%), a subset that was significantly lower in individuals with HIV (median, 20%; IQR, 15–32%), aTB (median, 16%; IQR, 4–19%), or both (median, 9%; IQR, 2.6–22%) (Figure 2B). In participants with HIV and/or aTB, IFN-γ^+IL-2^-TNF-α^+ cells counterweighed the reduction of triple-positive cells. Of note, unlike previously reported (2), no differences in the proportion of TNF-α single-positive Mtb-specific $CD4^+$ T cells were observed; these differences could arise from significant disparities in the age, ethnicity, and TB diagnosis in the study cohorts. Receiver operating characteristic curve analyses (Figure 2C) show that the proportion of IFN-γ^+IL-2^+TNF-α^+ or IFN-γ^+IL-2^-TNF-α^+ Mtb-specific $CD4^+$ T cells allowed the distinction between LTBI and aTB in HIV-uninfected individuals (AUC = 0.97 [$P < 0.0001$]; AUC = 0.92 [$P < 0.0001$], respectively), but not in HIV-infected persons.

In summary, these data show that HLA-DR expression on IFN-γ^+ Mtb-specific $CD4^+$ T cells represents a robust marker to distinguish between LTBI and aTB in both HIV-uninfected and antiretroviral therapy–naive HIV-infected individuals. This suggests that despite HIV-induced systemic immune activation, active bacterial replication promotes further up-regulation of HLA-DR on Mtb-specific $CD4^+$ T cells. On the contrary, the polyfunctional profile of Mtb-specific $CD4^+$ T cells associated with TB status solely in HIV-uninfected individuals, suggesting HIV infection may alter the secretion potential and/or localization of Mtb-specific $CD4^+$ T cells even in the absence of bacterial replication. One main limitation of such assays, requiring cell stimulation to identify Mtb-specific $CD4^+$ T cells, is that the analysis is restricted to individuals with detectable Mtb responses. Inclusion of additional immunodominant Mtb antigens could improve the "coverage" of Mtb responders. Further experiments will be needed to confirm these data in a larger study including HIV-infected participants receiving antiretroviral treatment. Nevertheless, this study confirms that HLA-DR expression could represent an important alternate tool to assess TB status in HIV-uninfected individuals and expand this finding to HIV-infected subjects. ■

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Improved Survival of Patients with Pulmonary Arterial Hypertension with *BMPR2* Mutations in the Last Decade

To the Editor:

Pulmonary arterial hypertension (PAH) is a rare, severe disease characterized by increased pulmonary arterial pressure due to abnormal intimal proliferation and resulting in elevated pulmonary vascular resistance and right heart failure. Over the past decade, several vasodilators have been developed for the