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MAJOR ARTICLE

Diminished *Mycobacterium tuberculosis*–specific T-cell Responses During Pregnancy in Women With HIV and Receiving Isoniazid Preventive Therapy

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Background. Pregnancy increases *Mycobacterium tuberculosis* (Mtb) reactivation risk and alters immune responses. We assessed Mtb-specific CD4+ T-cell responses in pregnant women with HIV (WLHIV) and without, including those receiving isoniazid preventive therapy (IPT).

Methods. We measured adaptive immune responses from 33 participants (HIV+ 21, HIV− 12) with positive interferon-gamma release assay during pregnancy (20–34 weeks' gestation), 6 weeks, and 12 months postpartum by intracellular cytokine staining. We measured overall responses using COMPASS and made comparisons by nonparametric analysis of variance.

Result. We observed diminished Mtb-specific CD4+ T-cell responses in WLHIV during pregnancy versus 12 months postpartum (COMPASS median functional score [FS] .009 vs 0.12, $P = .03$). WLHIV who received IPT (n = 8) during concurrent pregnancy had attenuated Mtb-specific CD4+ T-cell responses during pregnancy versus 12 months postpartum (median FS 8.3×10^{-7} vs 0.13, $P = .02$), but WLHIV who did not receive IPT during pregnancy had similar responses in pregnancy and postpartum. Mtb-specific CD8+ FS was increased postpartum in all groups. We found preexisting Mtb-specific CD4+ T-cell responses in participants who converted interferon-gamma release assay tests postpartum (n = 10).

Conclusions. Pregnant WLHIV, especially those on IPT, showed reduced Mtb-specific CD4+ T-cell responses. Understanding the impact of pregnancy on Mtb-specific T-cell responses may improve diagnostic approaches.

Keywords. HIV; isoniazid preventative therapy; LTBI; pregnancy; T cells; tuberculosis.

More than 1 million individuals died from tuberculosis (TB) in 2022 [1]. TB is associated with poor pregnancy outcomes for both mother and infant, including low birth weight, premature birth, and death [1–3]. Pregnant and early postpartum women are more likely to progress from latent TB infection to active TB disease, making this a potentially critical time to influence *Mycobacterium tuberculosis* (Mtb) [4, 5]. Our prior work, in a cohort of sub-Saharan African women enrolled in a HIV prevention trial, demonstrated that immune responses to TB were diminished during the third trimester of pregnancy [6]. In a separate cohort, Mtb-specific responses were diminished in pregnant women living with HIV (WLHIV), compared to pregnant women without HIV, but the prevalence of positive interferon-γ release assay (IGRA) results were similar regardless of HIV status [7]. Similarly, cohorts in India and Ethiopia demonstrated decreased IGRA sensitivity at delivery compared to early pregnancy and postpartum, with decreased Mtb-specific antigen responses among WLHIV in pregnancy and at delivery compared to women without HIV [8–11]. However, the effect of pregnancy on Mtb-specific T-cell responses remains somewhat uncertain [12].

WLHIV demonstrate altered adaptive immune responses overall and to Mtb infection [13], and Mtb-specific T-cell responses may be further influenced by the systemic immune modulation characteristic of pregnancy [14]. Among WLHIV, World Health Organization guidelines recommend isoniazid preventive therapy (IPT) to reduce the likelihood of Mtb progression including among pregnant WLHIV [15]. Recent studies suggest that IPT may also diminish Mtb-specific T-cell responses [16]. Understanding the impact of IPT and HIV on Mtb-specific

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immune responses in pregnant women may provide insight into how the Mtb-specific adaptive immune response develops and changes over time. In this study, we observed how HIV infection and IPT influenced Mtb-specific T-cell responses in pregnant and postpartum women.

T-cell responses are essential for Mtb control. Individuals with genetic deficiencies in the interferon γ (IFNγ) signaling axis develop disseminated mycobacterial infections, and individuals with acquired CD4+ T-cell deficiency have elevated risk of developing TB [13, 17]. IFNγ-independent T-cell responses also play a role in Mtb control. Interleukin-2 (IL-2), tumor necrosis factor (TNF), and IL-17–producing cells, CD4+ T cells that produce multiple cytokines simultaneously, CD8+ T cells, and central memory T cells all contribute to Mtb control [13, 18]. Our objective was to characterize the effect of pregnancy on Mtb-specific T-cell responses in women with and without HIV, and among WLHIV, the potential effect of IPT.

The Mother Infant Tuberculosis Infection incidence and Prevalence Study (MITIPS) was a cohort study that evaluated longitudinal TB infection detection using IGRA (newer-generation QFT-plus; Qiagen) and tuberculin skin test in pregnant WLHIV and women without HIV (HIV– women) in western Kenya, with follow-up to 1 year postpartum [7, 19]. Using samples from this study, we evaluated Mtb-specific T-cell phenotypes to assess the hypothesis that pregnancy, HIV, and IPT influence T-cell responses qualitatively and quantitatively that may reduce diagnostic accuracy of current testing approaches.

METHODS

Study Population

The protocol for this study was approved by the University of Washington Human Subjects Review Committee and Kenyatta National Hospital-University of Nairobi Ethics Review Committee. MITIPS was an observational study that enrolled pregnant WLHIV and HIV– women ≥16 years of age between 20–34 weeks' gestation at antenatal clinics in western Kenya. Details regarding the screening, enrollment, follow-up, and clinical procedures for the parent cohort have been previously published [19]. Participants were excluded if diagnosed with TB disease in the past year or found to have TB on enrollment. IGRA testing and peripheral blood mononucular cell (PBMC) collection was performed at enrollment during pregnancy, 6 weeks postpartum, and 12 months postpartum. Enrollment and IGRA testing began January 2018; PBMC collection on enrollment in pregnancy was initiated September 2018. A QFT-Plus TB 1 or TB 2 antigen response of ≥0.35 IU/mL (minus nil, with nil <8 IU/mL and positive mitogen control) was considered positive, per manufacturer recommendations [19]. Participants with HIV received IPT as part of routine HIV care outside of the study regardless of IGRA testing per Kenyan guidelines [20]. Those participants taking IPT and antiretroviral therapy (ART), either before this study or on study enrollment, continued these medications through the study period. Adults without HIV were not routinely eligible for IPT during the implementation of the parent study.

Our inclusion criteria were to include participants with persistent positive IGRA results in pregnancy and postpartum, with enrollment pregnancy samples collected during the second or third trimester of pregnancy, and available samples at both 6 weeks and 12 months postpartum. We excluded 1 participant who was diagnosed with active TB during postpartum follow up. We added 3 study groups. "Converters" were study participants with negative IGRA test during enrollment in pregnancy and positive tests either during the 6-week or 12-month postpartum visit. "Reverters" were participants with a positive IGRA test result at enrollment in pregnancy and negative postpartum. Last, we randomly identified 5 persistent IGRA-negative participants as a control to include in our analysis. Details of sample processing, reagents used, and data collection are in the Supplementary Methods.

Statistical Analysis

We used the R package COMPASS to analyze the cytokine response of CD4+ and CD8+ T cells. COMPASS uses a Bayesian hierarchical framework to model all observed cell subsets and selects those most likely to have antigen-specific responses [21, 22]. Using all collected flow cytometry data, COMPASS determines the likelihood of an antigen-specific response over background for any given cytokine-producing subset. COMPASS provides a functional score (FS), which is the proportion of antigen-specific subsets detected among all possible ones, creating a single number to capture the magnitude and breadth of the T-cell response. Our primary outcome of interest was the COMPASS FS from CD4+ and CD8+ T cells of IGRA-positive women, comparing Mtb-specific antigens ESAT-6 and CFP10-induced responses with negative control responses. We compared COMPASS FS in converters and reverters using Kruskal-Wallis tests followed by the Dunn comparison to assess any IFNγ-independent T-cell responses in these populations before IGRA conversion. Differences in median values were compared among timepoints of interest using Kruskal-Wallis tests as the primary analysis, followed by Dunn test to compare individual groups.

RESULTS

Baseline Cohort Characteristics

We studied a subset of HIV– women and WLHIV enrolled in the MITIPS cohort (n = 400 enrollees) that had peripheral blood collected longitudinally at 3 different time points: the second or third trimester (20–34 weeks' gestation), 6 weeks postpartum, and up to 12 months postpartum (n = 34 HIV– and n = 23 WLHIV). These individuals had IGRA tests that

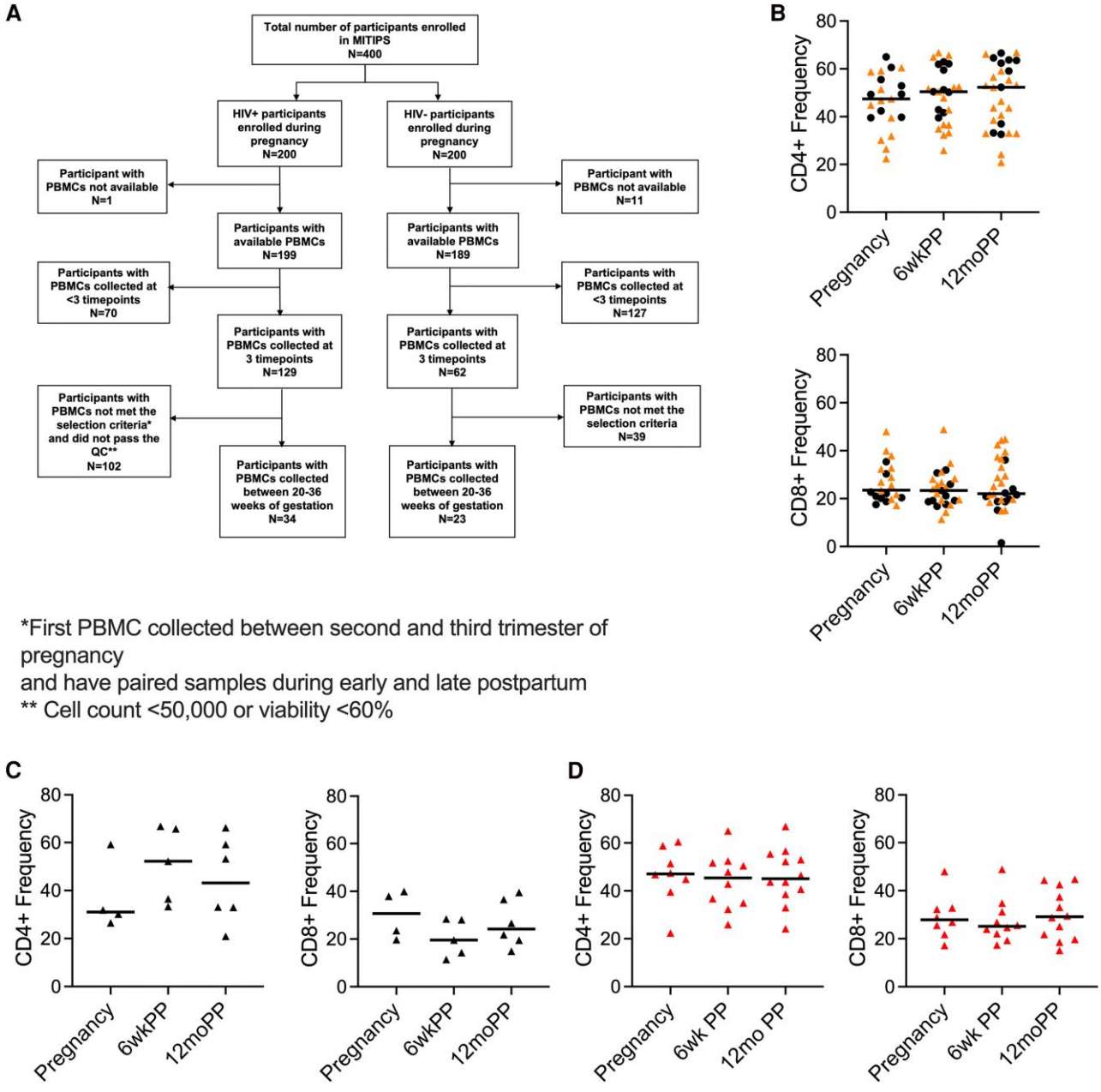

Figure 1. Study flowchart and baseline immune characteristics of study population. *A*, Flowchart demonstrating the sample selection process, for participants with persistently positive IGRA (N = 33, meeting study criteria). And additional subset of participants with persistently negative IGRA (n = 5), IGRA converters (n = 10), and reverters (n = 9) were evaluated. *B*, Baseline CD4+ and CD8+ T-cell proportion of persistently QFT-positive samples. n = 21 during pregnancy (pregnancy), n = 25 during 6 weeks postpartum (6wkPP), n = 28 during 12 months postpartum (12moPP). *C-D*, Baseline CD4+ and CD8+ frequencies in *C*, WLHIV receiving IPT during pregnancy and *D*, those who did not. N = 33. Black circles—HIV– study participants; gold triangles—WLHIV participants. Bar represents median value. Black triangles—WLHIV who received IPT during concurrent pregnancy. Red triangles—no concurrent IPT. Abbreviations: IGRA, interferon-γ release assay; IPT, isoniazid preventive therapy; WLHIV, women living with HIV.

were either consistently positive in pregnancy and postpartum (n = 33), consistently negative IGRA during pregnancy and postpartum (n = 5), or converted from a negative IGRA to positive postpartum (n = 10) or reversion (n = 9) from positive IGRA to a negative test postpartum (Figure 1*A*). Of these women, the median age of participants at enrollment was 24 years (interquartile range 21–30); median gestational age was 26 weeks (interquartile range 22–29). Among WLHIV, the median CD4 count was 557 cells/mm^3 and all were on ART at enrollment in pregnancy. Twenty-eight WLHIV received IPT before enrollment and 8 (28.6%) were on IPT as part of routine HIV care at their study enrollment in pregnancy. The baseline characteristics of study participants stratified by their HIV infection status, is shown in Table 1. CD4+ and CD8+ T-cell

Table 1. Study Participant Characteristics

Characteristic	Overall Median (IQR) or n (%) N = 57	WLHIV Median (IQR) or n (%) N = 34	HIV Negative Median (IQR) or n (%) N = 23	RR[a] (95% CI)	*P*
Age (y)	24 (21–30)	26.5 (22–32)	23 (21–25)	1.04 (1.01–1.08)	.013
Gestational age	26 (22–29)	24 (21–29)	27 (23–30)	0.97 (.94–1.01)	.167
WLHIV		34 (59.7)			
CD4 count (cells/mm^3), N = 14	…	557 (391–760)	…	–	–
Viral load undetectable,[b] N = 31	…	26 (83.9)	…	–	–
HIV VL at enrollment (copies/mL)	…	0 (0–54)	…	…	
ART initiation before pregnancy, N = 34	…	22 (64.7)	…	–	–
TB history					
History of TB	1 (1.8)	1 (2.9)	0	–	1.000[c]
History of IPT use, N = 34	…	28 (82.4)	–	–	–
IPT on enrollment, N = 28	…	8 (28.6)	–	–	–
Samples	N = 171	N = 102	N = 69	…	
Pregnancy stage					
Pregnancy	57 (33.3)	34 (33.3)	23 (33.3)	…	
2nd trimester (14 to <28 wk)	29 (50.9)	19 (55.9)	10 (43.5)	…	
3rd trimester (≥28 wk)	28 (49.1)	15 (44.1)	13 (56.5)	…	
Postpregnancy	114 (66.7)	68 (66.7)	46 (66.7)	…	

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IGRA, interferon-gamma release assay; IPT, intermittent preventive therapy; IQR, interquartile range; RR, relative risk; TB, tuberculosis; VL, viral load; WLHIV, women living with HIV.

[a]RR estimated using a generalized linear model with log link and Poisson family.

[b]Viral load undetectable <20 copies/mL.

[c]Fisher exact test.

frequency was similar between HIV– women and WLHIV during pregnancy and postpartum time points (Figure 1*B*, flow cytometry gating in Supplementary Figure 1) and was similar between WLHIV receiving IPT (Figure 1*C*) and those who did not receive IPT during the study period (Figure 1*D*).

Mtb-specific T-cell Responses Were Diminished in Pregnant WLHIV Compared to Postpartum

Mtb-specific IFNγ-producing CD4+ cells and IFNγ-independent T-cell responses are both important for Mtb control [23]. Therefore, we investigated CD4+ and CD8+ Mtb-specific T-cell responses incorporating IFNγ, IL-2, and TNF responses in pregnant women including WLHIV and those receiving concurrent IPT. Among all participants with persistently positive IGRA, overall Mtb-specific CD4+ T-cell FS were similar between pregnancy and postpartum (Figure 2*A*). Stratifying responses by study participant HIV infection status, HIV-negative participants exhibited consistent Mtb-specific T-cell responses across all time points (Figure 2*B*). In contrast, WLHIV demonstrated significantly diminished Mtb-specific CD4+ T-cell FS during pregnancy compared to 12 months postpartum (Figure 2*C*; median FS 0.009 vs 0.12, $P = .03$, Kruskal-Wallis test). We noted fewer multifunctional and polyfunctional T-cell populations in WLHIV during pregnancy (Supplementary Figure 2A) compared to either postpartum time point (Supplementary Figure 2B-C). We found no difference in Mtb- specific CD4+ T-cell cytokine production in the group with persistently negative IGRA test during pregnancy or postpartum (data not shown). We did not detect any difference in CD4+ T-cell responses stimulated with the nonspecific T-cell activator PMA/ionomycin (Supplementary Figure 2D) at any time studied. In summary, among WLHIV with persistently positive IGRA, Mtb-specific T-cell responses are diminished during the second and third trimesters of pregnancy compared to postpartum.

Mtb-specific T-cell Responses Were Diminished in Pregnant WLHIV, Especially Those Receiving IPT

World Health Organization guidelines recommend IPT for individuals living with HIV residing in high TB burden countries, including during pregnancy [15]. We measured the impact of IPT on Mtb-specific T-cell responses in pregnant WLHIV. FS were diminished during pregnancy among WLHIV taking IPT during the current pregnancy compared to postpartum (Figure 2*C*; median FS 8.3×10^{-7} during pregnancy, 0.09, 6 weeks postpartum, and 0.13 12 months postpartum, $P = .02$; Kruskal-Wallis test). We found no significant difference in Mtb-specific T-cell responses during pregnancy and postpartum periods in WLHIV who never received IPT or finished IPT before the current study enrollment (Figure 2*D*).

Mtb-specific CD8+ T-cell Responses Increased Postpartum in WLHIV

We evaluated Mtb-specific CD8+ T-cell responses given their importance in Mtb control and to see if these responses were

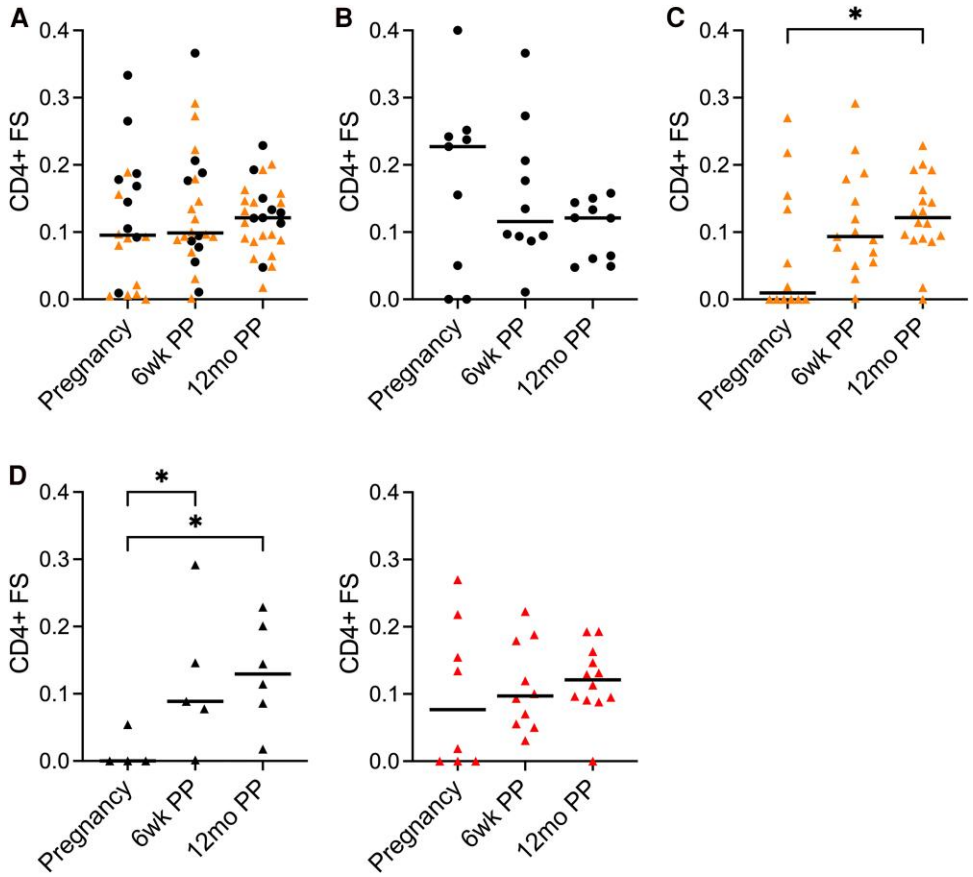

Figure 2. Mtb-specific CD4+ T-cell responses are diminished in WLHIV during pregnancy, especially among those receiving IPT. *A-C*, Mtb-specific CD4+ T-cell functional scores (FS) were measured by COMPASS: *A*, overall, in *B*, HIV− participants, and *C*, WLHIV. *D*, Mtb-specific CD4+ T-cell FS in WLHIV receiving IPT during the current pregnancy (*black triangle*) and those who did not (*gray triangles*). Black circles and gold triangles indicate HIV-negative and HIV-positive participants, respectively. Bar indicated median values. *$P < .05$, Kruskal-Wallis test, column to column comparisons are adapted from Dunn test. Abbreviations: IPT, isoniazid preventive therapy; WLHIV, women living with HIV.

differentially impacted from CD4+ T-cell responses [24, 25]. Mtb-specific CD8 T-cell FS were diminished overall during pregnancy compared to late postpartum in participants with persistently positive IGRA (Figure 3*A*, median FS 0.0001, pregnancy and 6 weeks postpartum; 0.02, 12 months postpartum; $P < .0001$). These results were similar in HIV− women (Figure 3*B*), WLHIV (Figure 3*C*), and those receiving IPT (Figure 3*D*). We did not detect any Mtb-specific IFNγ+ or polyfunctional CD8+ T-cell responses in this population during pregnancy and 12 months postpartum (Supplementary Figure 3A-D).

Nonspecific T-cell Activation was Increased Postpartum Among HIV− Study Participants but Not WLHIV

Nonspecifically activated HLA-DR+CD38+CD4+ T cells are a correlate of risk for progression from latent TB infection to active TB disease. We found increased proportions of HLA-DR +CD38+ CD4 T cells in the early postpartum period in HIV− women (Figure 4*A-C*, $P = .02$; flow minus one control samples, Supplementary Figure 4A) but not in WLHIV. Among WLHIV, we found no differences based on IPT administration at any time points (Figure 4*D*).

IGRA "Converters" Demonstrate Mtb-specific T-cell Responses During Pregnancy

Recent studies suggest that T-cell responses are dynamic after Mtb exposure [26, 27]. A subset of study participants developed positive IGRA tests during either early or late postpartum ("converters") after initial negative IGRA results at enrollment in pregnancy. We assessed whether these individuals demonstrated preexisting IFNγ-independent T-cell responses in pregnancy before IGRA conversion. Overall FS were lower in IGRA converters than in those individuals with persistently positive IGRA, and FS were similar before and after IGRA conversion in this population (Figure 5*A*; FS). We noted dynamic shifts in the specific cytokines detected over time. During pregnancy, we found increased IL-2+ CD4+ T-cell frequency with few IFNγ-positive results (Figure 5*B*). Postpartum, TNF+ and IFNγ+ cell frequencies increased, with the highest responses measured 12 months after pregnancy (Figure 5*B*). Among converters, we noted increased

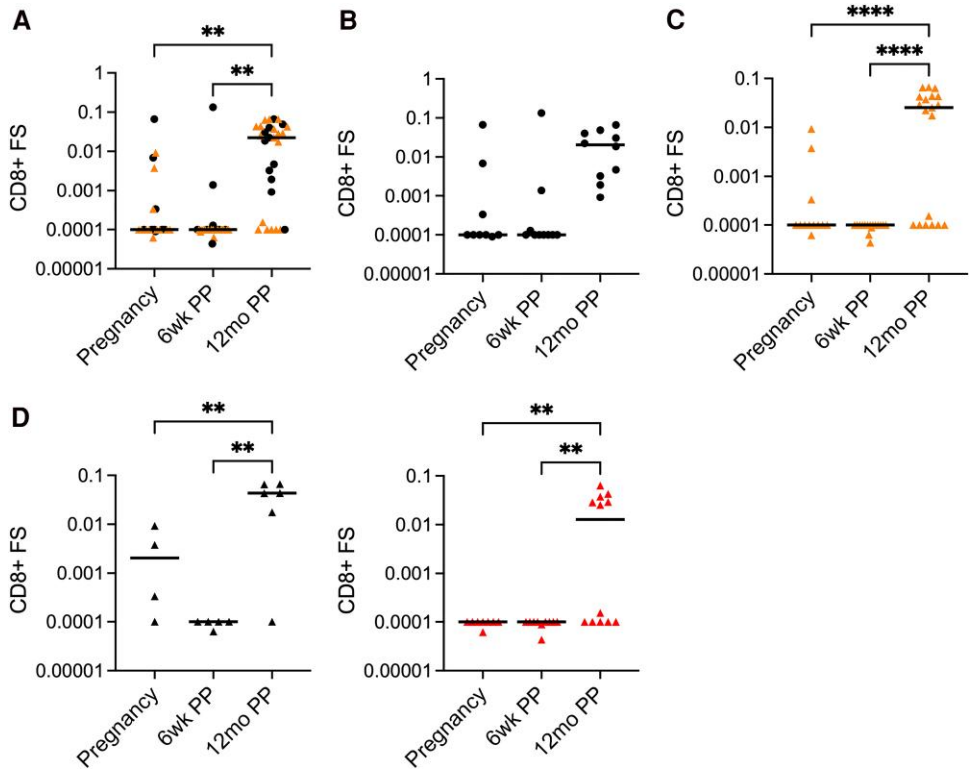

Figure 3. Mtb-specific CD8+ T-cell responses increase postpartum in women with and without HIV. *A-B*, Mtb-specific CD8+ T-cell functional scores (FS) were measured by COMPASS in *A*, overall, *B*, HIV– participants, and *C*, WLHIV. *D*, Mtb-specific CD8+ T-cell FS in those receiving IPT during the current pregnancy (black triangles) and those who did not (gray triangles). Black circles and gold triangles indicate HIV-negative and HIV-positive participants respectively. Bar indicated median values. $^{*}P < .05$, Kruskal-Wallis test, column to column comparisons are adapted from Dunn test. Abbreviation: Mtb, *Mycobacterium tuberculosis*.

frequencies of nonspecifically activated HLA-DR+CD38+ CD4+ T cells during pregnancy compared with 6 weeks and 12 months postpartum (Figure 5*C*).

Dynamic T-cell cytokine responses may be accompanied by alterations in cell surface markers of memory T-cell homing, therefore we evaluated CD45RA and CCR7 expression to further distinguish distinct T-cell memory phenotypes [28]. We found increased proportions of Mtb-specific central memory CD4 T cells (CD45RA-CCR7+) and fewer naïve CD4+ T cells (CD45RA + CCR7+) during pregnancy in converters (Figure 5*D*; $P = .01$ and .03, respectively) compared to postpartum. We did not observe any variation in CD45RA-CCR7– T effector memory populations. We measured expression of central memory, effector memory, and naïve cells based on CCR7 and CD45RA expression in persistently IGRA-positive study participants, but no significant differences were noted in this population (data not shown). Overall, these data find that some individuals that convert their IGRA tests postpartum demonstrate evidence of prior Mtb exposure with a negative IGRA test.

Mtb-specific IL-2+ CD4+ T-cell Responses Persist in "Reverters"

We also observed the characteristics of the T-cell response in participants that lost IGRA positivity after pregnancy ("reverters"). As with converters, we found no changes in the FS score during or after pregnancy (Figure 6*A*). We found the proportion of IL-2+CD4+ T cells was stable during pregnancy and postpartum, whereas the proportion of TNF+ and IFNγ+ CD4+ T cells decreased postpartum (Figure 6*B*, $P = .03$ and 0.02, respectively). Evaluation of COMPASS heatmaps revealed low-frequency positivity that persisted after pregnancy in a subset of participants (Supplementary Figure 5A-C). We did not detect changes in the frequencies of nonspecifically activated HLA-DR+CD38+ CD4+ T cells during pregnancy compared with 6 weeks and 12 months postpartum (Figure 6*C*), nor did we detect significant differences in the proportion of memory T-cell populations in pregnancy compared to postpartum time points (Figure 6*D*). Therefore, we were able to detect modest Mtb-specific T-cell responses in the reverter population.

DISCUSSION

Mtb-specific T-cell responses were diminished in pregnant WHIV, especially in those receiving IPT. CD4+ T-cell responses rebounded postpartum in WLHIV, whereas CD8+ responses increased in both HIV– and WLHIV groups postpartum. In

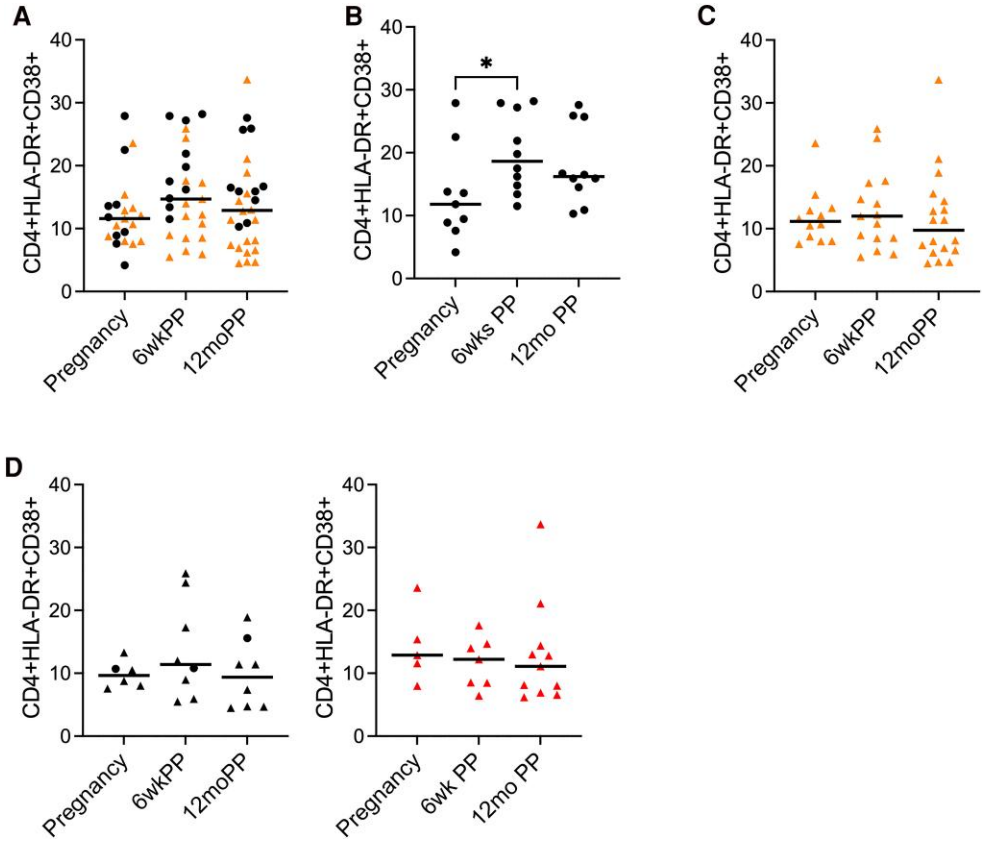

Figure 4. Nonspecifically activated CD4+ T cells increase in the early postpartum period in HIV– women. *A-D*, The proportion of nonspecifically activated (HLA-DR+CD38+) CD4+ T cells stratified by pregnancy and postpartum period: *A*, overall (n = 28), *B*, HIV-negative individuals (black circles) and *C*, in WLHIV (gold triangles). *D*, WLHIV who received IPT during current pregnancy (black triangles) or whose who did not (gray triangles). Bar indicates median values. $^*P < .05$, Kruskal-Wallis test, column to column comparisons are adapted from Dunn test. Abbreviations: IPT, isoniazid preventive therapy; WLHIV, women living with HIV; Mtb, *Mycobacterium tuberculosis*.

preliminary studies, we found that IGRA testing may not fully capture Mtb exposure in a population of pregnant women.

Pregnant WLHIV had diminished Mtb-specific CD4+ T-cell responses that rebounded in the postpartum period, alongside Mtb-specific CD8+ T-cell responses in both HIV– participants and WLHIV. We noted decreased CD4+ FS in WLHIV receiving IPT during pregnancy compared to postpartum. In a subset of participants with negative IGRA during pregnancy, we demonstrated direct evidence of IFNγ-independent Mtb-specific T-cell responses and characteristic shifts in memory T-cell surface markers, suggesting diminished IGRA responses during pregnancy despite evidence of prior sensitization. Last, we noted an increase in nonspecific T-cell activation associated with TB progression in other populations was increased postpartum among HIV– study participants but not WLHIV.

WLHIV demonstrated diminished Mtb-specific T-cell responses during pregnancy. These results were consistent with a recent study of the same population, measuring quantitative IGRA results [19] in addition to studies of qualitative IGRA positivity [10, 29]. However, in a recent study of Ethiopian women, increased Mtb-specific immune responses were noted during pregnancy compared with postpartum [12]. Several differences may potentially lead to these differences and may shed insight into Mtb pathogenesis in pregnant women. First, the timing of blood collection during pregnancy is critical. We observed the strongest differences in immune responses during the late third trimester. Second, immune modulation is cytokine-specific. We found the largest differences in Mtb-specific IFNγ responses and overall via COMPASS. Sample collection and storage may play a role in response; samples from whole-blood supernatants and PBMC may induce distinct cytokine responses. The HIV status of participants influences the magnitude of pregnancy-induced immune modulation and not all studies included both women with and without HIV [12]. Median immune responses were diminished in WHIV both compared to HIV– women and compared to later postpartum in multiple prior studies [7, 19]. Overall, study timing, measurements, and comorbidities may play a key role in the immune modulation observed in pregnant women and may explain differences in study observations.

In addition to diminished CD4+ T-cell responses, we found alterations in CD8+ T-cell responses and nonspecific T-cell

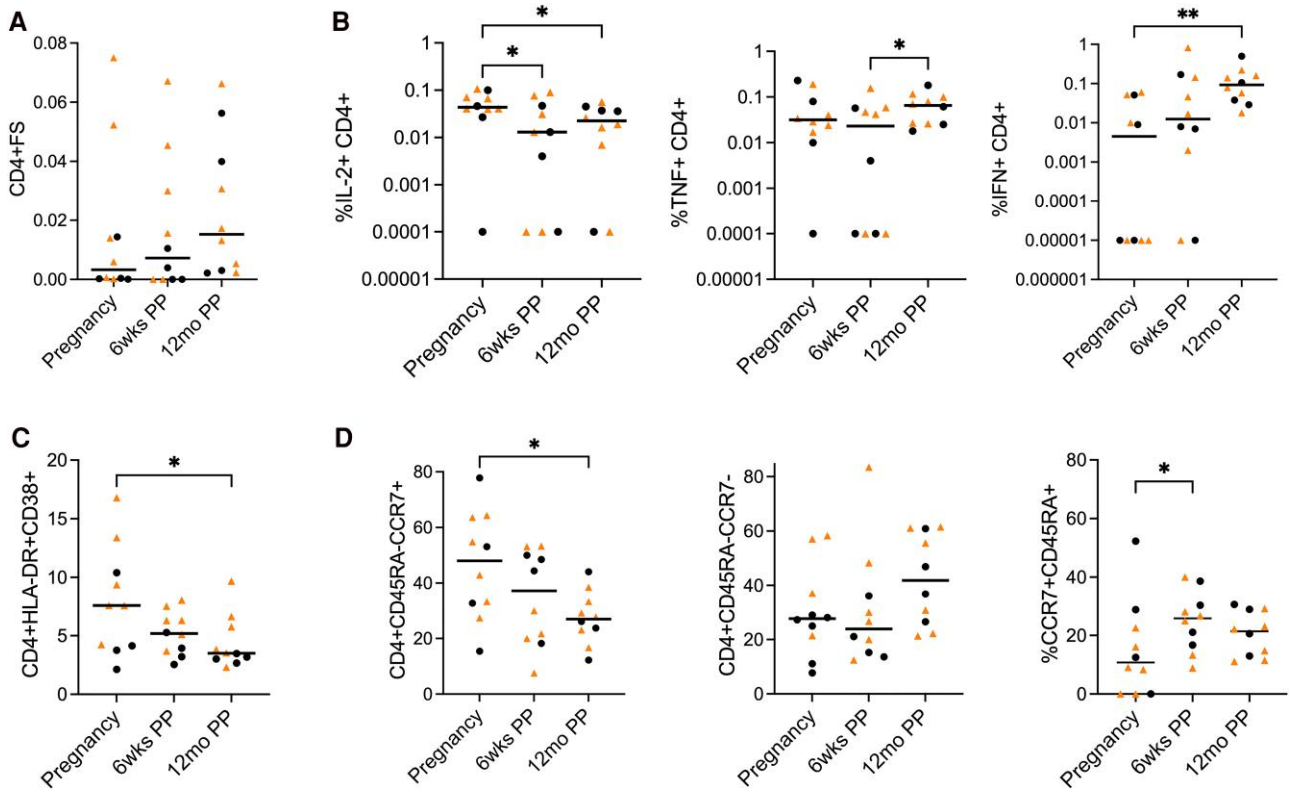

Figure 5. Mtb-specific T-cell responses were detectable in pregnant women who converted their interferon gamma release assay (IGRA) tests postpartum. *A*, Mtb-specific CD4+ T-cell FS in WLHIV and HIV– study participants with negative IGRA in pregnancy with later conversion, n = 10. *B*, Proportion of single cytokine response during pregnancy and postpartum. *C*, Proportion of HLA-DR+CD-38+ CD4+ T cells during pregnancy and postpartum period. *D*, Overall proportion of any cytokines producing effector memory (CD45RA-CCR-), central memory (CD45RA-CCR7+), and naïve (CD45RA + CCR7+) CD4+ T-cell phenotype by pregnancy status. Black circles and gold triangles indicate HIV-negative and HIV-positive participants respectively, bar represents median value. The Dunn test was used to do column-to-column comparison. Abbreviations: FS, functional score; WLHIV, women living with HIV.

activation that support this observation. Mtb-specific CD8+ T-cell responses are important for control and recognize macrophages containing replicating Mtb [23, 24, 30]. We observed expanded Mtb-specific CD8+ T cells postpartum. As this occurs after diminished CD4+ responses, our observations support the hypothesis that CD8+ cells expand to control expanding Mtb populations from pregnancy. Similarly, we confirmed our prior finding of increased nonspecific T-cell activity early postpartum in HIV– study participants. Increased nonspecific T-cell activation is a marker for Mtb progression, so increases in this population may represent some degree of loss of Mtb control [31]. Future studies will investigate more fully the cellular populations and interactions required for host defense during pregnancy and postpartum.

How HIV influences Mtb-specific immune responses in pregnant women, even in women without overt evidence of immune suppression, remains unclear. HIV may remain active in reservoir areas of the body that contribute to T-cell activation but are absent in the peripheral blood [32]. Chronic immune activation associated with ongoing HIV infection may influence functional T-cell responses to antigen, and this response may interact with immune changes from pregnancy to further reduce T-cell responses [28]. Prior immune scarring resulting from clonal T-cell destruction may influence future immune responses [33]. Nonetheless, the magnitude of Mtb-specific T cell responses during pregnancy are diminished, suggesting decreased sensitivity of IGRA testing in pregnant women, especially WLHIV.

IPT was associated with diminished Mtb-specific T-cell responses among pregnant WLHIV. Why this occurs is uncertain, but mycobacteria-specific CD4+ T-cell immune responses are enhanced by the ongoing presence of live mycobacteria [34]. IPT may kill remaining live mycobacteria and diminish T-cell responses to mycobacteria. IPT treatment reduced transcriptional profiles associated with subclinical TB in a Kenyan cohort and IFNγ production in Mtb-stimulated peripheral blood drawn from household contacts of individuals with confirmed pulmonary TB [26, 27]. These responses rebounded postpartum, which may be due to changes from pregnancy on T cell directly or by the cessation of IPT and subsequent exposure to Mtb within the community or household.

We found preexisting Mtb-specific CD4+ T-cell memory responses that were independent of IFNγ in IGRA-negative

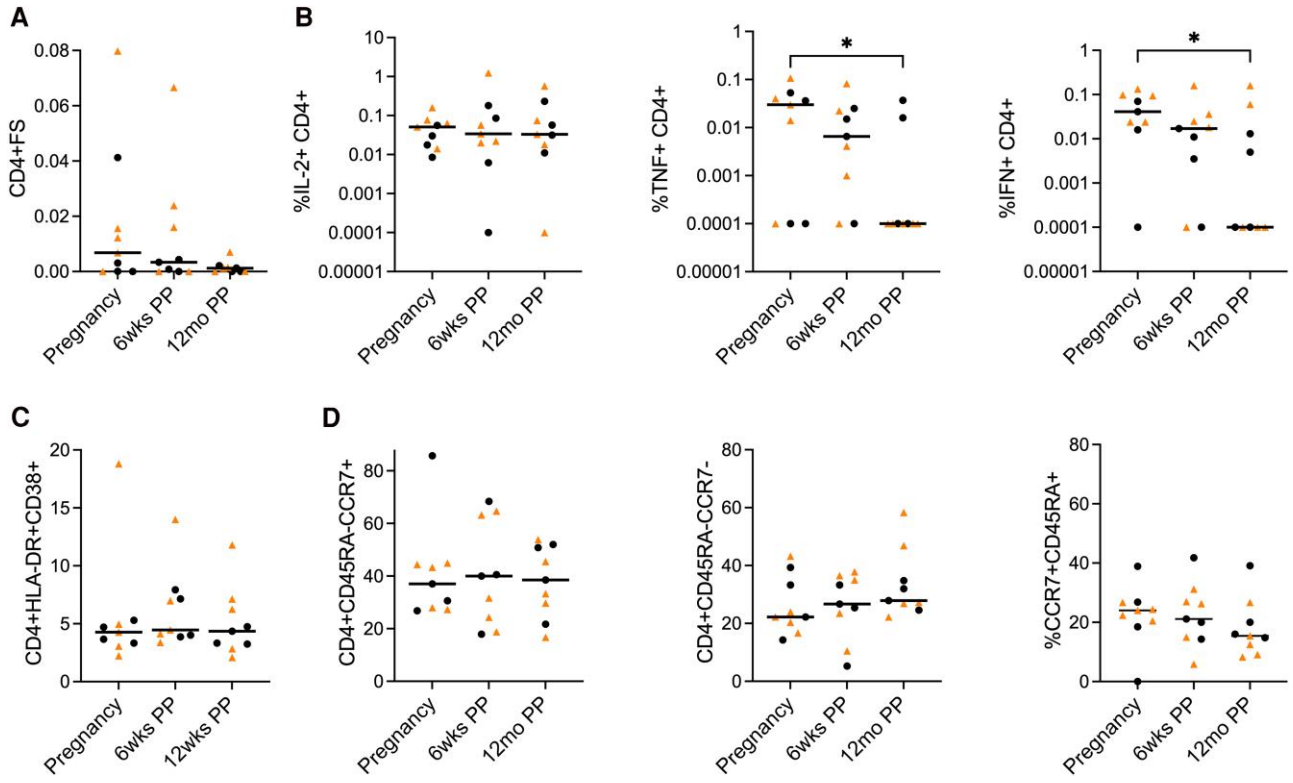

Figure 6. Mtb-specific immune responses diminish selectively in individuals that revert from IGRA positive to negative postpartum. *A*, Mtb-specific CD4+ T-cell FS in WLHIV and HIV– study participants with positive IGRA in pregnancy with later reversion, n = 9. *B*, Proportion of IL-2+, TNF+, and IFNγ+ CD4+ T cells during pregnancy and postpartum. *C*, Proportion of HLA-DR+CD38+ CD4+ T cells during pregnancy and postpartum period. *D*, Overall proportion of any cytokines producing effector memory (CD45RA-CCR-), central memory (CD45RA-CCR7+), and naïve (CD45RA+CCR7+) CD4+ T-cell phenotype by pregnancy status. Black circles indicate HIV– participants; gold triangles indicate WLHIV. Bar represents median value. Statistical significance measured by Dunn test. Abbreviations: FS, functional score; IGRA, interferon-gamma release assay; IFN, interferon; IL, interleukin; Mtb, *Mycobacterium tuberculosis*; TNF, tumor necrosis factor; WLHIV, women living with HIV.

pregnant women that converted their IGRA-positive postpartum. These data provide further evidence that pregnancy and other factors alter the functional T-cell response. Individual responses to IGRA tests vary over time, including in pregnant women [35]. Our data provide further evidence that Mtb-specific responses in pregnant women may be directly related to alterations in the immune response. Prior studies indicate that T-cell memory responses in pregnant women may increase postpartum but were hampered by the possibility that Mtb exposure could have occurred in the postpartum period as well.

Reversion of tuberculin skin tests is associated with reduced risk for TB disease [35]. It is unclear whether these individuals are at lower risk for developing TB. In prior studies, individuals that revert their IGRA tests develop immune response characteristics consistent with controlled Mtb infection [35]. In our study, reverters mimicked these findings, including ongoing active T-cell responses with decreased IFNγ production and maintenance of Mtb-specific T-cell memory without changes in nonspecific T-cell activation. These data suggest that loss of IFNγ responses in pregnancy do not mean the loss of T-cell memory responses overall.

Our study has several strengths. The MITIPS study longitudinally monitored pregnant women with and without HIV postpartum for 24 months, permitting us to observe the restoration of basal homeostasis of the immune response after birth and tissue repair associated with delivery. We leveraged the fact that WLHIV enrolled in this study were receiving ART with some receiving IPT during pregnancy, making their treatment relevant for contemporary populations in sub-Saharan Africa. We were able to correlate findings made via flow cytometry with concurrent IGRA testing, providing clinical laboratory correlation of our research findings. We used COMPASS, a statistical tool for merging multiple distinct cytokine responses into an overall number, which provided a sensitive measure to evaluate overall responses and increasing the power of the study to detect differences in adaptive immune responses.

This study also has several limitations. We did not detect diminished responses in HIV– pregnant women in our current study as we had in prior work [6]. However, the timing of sample collection during pregnancy was different between these studies, with blood collection in this study occurring at 34 weeks' gestation at the latest, whereas our prior evaluation

evaluated participants with blood collected up to 40 weeks' gestation, when immune changes in pregnancy may be most pronounced [6, 10]. Additionally, although we observed associations between IPT and diminished Mtb-specific T-cell responses, sample sizes were small. These issues require larger studies to make firm conclusions about their role in Mtb immune responses. We could not fully control for the potential confounding effect of HIV severity among participants, partly because of variation in the timing of ART initiation. However, we did not note differences in basal proportion of CD4 and CD8+ T-cell populations between groups. We did not, however, note differences in basal proportion of CD4 and CD8+ T-cell populations between groups in this study. Women may have been exposed to Mtb postpartum or during pregnancy and may have developed subclinical disease that was not manifested during the study period. However, they were monitored for 24 months after study enrollment, and only 1 woman developed active TB, suggesting this was a rare outcome. The numbers of study participants who converted their IGRA postpartum or reverted to a negative IGRA were too small to draw conclusions on their own. However, these data provide additional evidence that IGRA testing may not fully identify individuals exposed to Mtb.

Overall, we detected diminished Mtb-specific T-cell responses in pregnant WHIV that increased postpartum and was exacerbated in WLHIV taking IPT. Women with negative IGRA tests during pregnancy but converted postpartum demonstrated evidence of preexisting IFNγ-independent T-cell responses suggesting that tests reliant of IFNγ to detect Mtb infection could be lower during pregnancy. These findings suggest further that immune testing for Mtb exposure during pregnancy has diminished effectiveness and provides evidence for ongoing TB preventive efforts during pregnancy.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. Conceived and designed the analysis: J.A.S., S.M.L., G.J.S., A.S. Collected the data: A.S., J.S.E., J.M., E.M.O., D.M., J.K. Contributed data or analysis tools: B.A.R., G.J.S., T.L., J.M., E.M.O. Performed the analysis: A.S., J.N.E., T.L., B.A.R., S.M.L., J.A.S. Wrote the paper: A.S., J.N.E., S.M.L., J.A.S.

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Potential conflicts of interest. The authors declare that they do not have any association that might pose a conflict of interest.

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