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SUPPLEMENT ARTICLE

T-Cell Homeostatic Imbalance in Placentas From Women With Human Immunodeficiency Virus in the Absence of Vertical Transmission

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Background. Implementation of universal antiretroviral therapy (ART) has significantly lowered vertical transmission rates but has also increased numbers of human immunodeficiency virus (HIV)–exposed uninfected children, who remain vulnerable to morbid effects. In the current study, we investigated whether T-cell alterations in the placenta contribute to altered immune status in HIV-exposed uninfected.

Methods. We analyzed T cells from term placenta decidua and villous tissue and paired cord blood from pregnant women living with HIV (PWH) who initiated ART late in pregnancy (n = 21) with pregnant women not living with HIV (PWNH) (n = 9).

Results. Placentas from PWH showed inverted CD4/CD8 ratios and higher proportions of tissue resident $CD8^+$ T cells in villous tissue relative to control placentas. $CD8^+$ T cells in the fetal capillaries, which were of fetal origin, were positively correlated with maternal plasma viremia before ART initiation, implying that imbalanced T cells persisted throughout pregnancy. In addition, the expanded memory differentiation of $CD8^+$ T cells was confined to the fetal placental compartment and cord blood but was not observed in the maternal decidua.

Conclusions. T-cell homeostatic imbalance in the blood circulation of PWH is reflected in the placenta. The placenta may be a causal link between HIV-induced maternal immune changes during gestation and altered immunity in newborn infants in the absence of vertical transmission.

Keywords. CD4; CD8; T cells; placenta; HIV; HEU; HIV-exposed; placenta pathology; villous tissue.

In adults, human immunodeficiency virus (HIV) causes severe immune dysregulation, characterized by systemic depletion of $CD4^+$ T cells, increased HIV-1–specific $CD8^+$ T cells, inflammation and a progressive failure of the immune system [1–3]. Initiation of antiretroviral therapy (ART) has been shown to augment HIV-specific $CD4^+$ T-cell responses, but normalization of the CD4/CD8 T-cell ratio does not occur in a large proportion of people with HIV [4]. In pregnant women living with HIV (PWH), there is evidence that women who start ART before pregnancy are more likely to have adverse birth outcomes than those who start ART during pregnancy [5].

Placentas from PWH exhibit increased signs of inflammation and injury affecting maternal vasculature and circulation [6, 7]. Studies also show that using protease inhibitor–based ART during pregnancy is associated with placental injury affecting maternal vascularization and impaired decidualization [8, 9]. In addition, although maternal and fetal circulation within the placenta takes place in distinct compartments, there is evidence that maternal HIV and viral load affects the fetal immune system. HIV-exposed uninfected children (HEU) have been shown to have lower $CD4^+$ T-cell counts and CD4/CD8 T-cell ratios at birth [10, 11], and this appears to be related to maternal viral loads >1000 RNA copies/mL [12]. HIV-unexposed uninfected children (HUU) have an almost completely naive T-cell repertoire, but at birth HEU can have increased proportions of differentiated immune cells, suggestive of antigen experience in utero [10, 13]. Indeed, a number of factors including impaired thymic output and functioning may underlie the immune alterations in HEU [14, 15]. Here, we sought to investigate how HIV exposure in utero may contribute to altered HEU immunity [16, 17].

To test the hypothesis that maternal HIV infection is associated with disruption of T-cell homeostasis in the placenta and cord blood from HEU newborns, we examined term placentas

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from PWH from a randomized trial in pregnant mothers starting dolutegravir (DTG) versus efavirenz (EFV)–containing therapy in the third trimester (DolPHIN-2; NCT03249181) [18], as well as pregnant women not living with HIV (PWNH), as controls. We show that placentas from PWH have inverted CD4/CD8 ratios with higher $CD8^+$ T-cell counts in villous tissue relative to control placentas, contributing to T-cell homeostatic imbalance in the placenta at birth.

METHODS

Cohort

We included 21 placentas with 9 paired cord blood samples from PWH and HEU and 9 placentas from PWNH with 5 cord blood samples from HUU infants in this study. The PWH group was nested in the DolPHIN-2 study recruited from the Gugulethu Community Health Centre, Cape Town, South Africa [18]. PWNH were enrolled from Khayelitsha Site B Midwife Obstetric Unit, Cape Town. All placentas were from term deliveries (>37 weeks' gestation).

Clinical Data Collection

As part of DolPHIN-2, maternal systemic $CD4^+$ T-cell counts and plasma viral loads copies were measured at ART initiation at 28 weeks' gestation (visit 1), at 29 (visit 2), 33 weeks (visit 3), and 36 weeks (visit 4), and at day 14 after delivery (visit 5). The level of HIV-1 RNA detection was 50 copies/mL [18].

Placenta and Cord Blood Processing

Cells were isolated from each placenta, as described elsewhere [19] and illustrated in Supplementary Figure 1. Placentas were collected in RPMI 1640 supplemented with 10% fetal calf serum and penicillin-streptomycin at room temperature, and processing was performed within 6 hours after delivery. Each placenta was dissected to obtain the decidua parietalis, basalis, and villous tissue. Enzymatic lymphocyte isolation was performed using collagenase I and DNAse I. The lymphocyte fraction was obtained after Percoll density centrifugation and incubated with violet amine reactive viability dye (VIVID; Thermofisher). The cells were then fixed using BD FACS lysing solution and cryopreserved in liquid nitrogen until analysis. Cord blood mononuclear cells were isolated on Ficoll, fixed, and cryopreserved until analysis.

Placental Pathology

Whole placentas were fixed in 10% buffered formalin before histopathology. Specimens were macroscopically examined, and samples from the umbilical cord, placental membranes and placental disk were obtained based on the Amsterdam Placental Workshop Group consensus statement [20]. Briefly, 4 blocks were prepared from each placenta, including a roll of the placental membranes, 2 cross-sections of the umbilical cord, and full-thickness sections of the placental parenchyma, and examined in detail, as described elsewhere [21].

Flow Cytometry

Placental and cord blood cells were labeled with fluorochrome-conjugated monoclonal antibodies: CD3 (clone UCHT1), CD4 (clone SK3), CD8 (clone SK1), CD45RA (clone H100), CD28 (clone CD28.2), CD14 (clone MHCD1417), and CD45 (clone MHCD4530). Samples were acquired using an LSR II flow cytometer (BD Biosciences). Total $CD4^+$ and $CD8^+$ T cells were expressed as proportions of $CD3^+$ T cells (Supplementary Figure 2).

Immunohistochemistry

Formalin-fixed paraffin-embedded placenta tissue blocks were cut into 5-µm sections and stained with CD8 (clone C8/144B), with tonsillar tissue serving as a control. Briefly, the slides were baked overnight at 56°C, rehydrated in xylene followed by varying concentrations of alcohol, and then incubated in 3% hydrogen peroxide. Heat-mediated antigen retrieval was performed using an ethylenediaminetetraacetic acid buffer (pH 9). The slides were then incubated with 1% bovine serum albumin and stained with anti-CD8. The images were acquired on Zeiss Axioskop 200 upright fluorescence microscope with an AxioCam high-resolution color camera.

Fluorescence In Situ Hybridization

Because there were 5 male infants, we selected those placental samples to identify the origin of the infiltrating lymphocytes by looking for the Y chromosome, using XY fluorescence in situ hybridization (FISH). Briefly, the slides were baked at 90°C for 15 minutes, deparaffinized in xylene, dehydrated in 100% ethanol and then placed in 10 mmol/L citric acid (pH 6.0). The slides were then dehydrated in varying concentrations of ethanol (70%, 85%, and 100%). We then applied a working solution of DXZ1/DYZ3 (Abbott Laboratories) to the target areas, codenatured with a ThermoBrite system (Abbott Laboratories), and hybridized overnight at 37°C. The slides were then counter stained with 4'-6'-diamidino-2-phenylindole (DAPI) (Vector Laboratories). Tissue samples were scanned and the qualitative result was determined based on observed signal patterns with CytoVision (Leica Biosystems).

Statistical Analysis

All flow cytometric data were analyzed using FlowJo software (version 10; TreeStar). Statistical analyses were performed using Prism (version 8; GraphPad Software), Stata (version 12.0; StataCorp), or R [22] software. Immunohistochemistry cell counts were performed using ImageJ Fiji software version 2 (developed by W. S. Rasband, National Institutes of Health). Tests of significance were performed using Mann-Whitney *U* and Kruskal-Wallis tests for intergroup comparisons. The associations between cell proportions and maternal viral load or $CD4^+$ T-cell counts were assessed using simple linear regression. All bivariate analyses including maternal and infant characteristics, and placental pathologic conditions stratified by

HIV exposure or by ART regimen were compared using χ^2 or Fisher exact tests and Wilcoxon rank-sum tests.

Study Approval

The study protocol, informed consent and all data collection tools were approved by the Human Research Ethics Committee of the University of Cape Town (no. 096/2017). Written and signed informed consent was obtained from all participants, including collection of placentas before study inclusion.

RESULTS

Participant Characteristics

Maternal and newborn infant characteristics are shown in Table 1. No significant differences in maternal age were noted between PWNH and PWH at enrollment, with the median gestational ages being 30 and 28 weeks, respectively, in the groups. PWH were more likely to be multigravida ($P = .003$). The median gestational age at delivery was 40 weeks in the PWNH and 39 weeks in the PWH ($P = .03$), and there was a lower birthweight trend in HEU infants ($P = .07$). We collected placentas from 21 PWH: 16 (76.2%) receiving EFV (with tenofovir disoproxil fumarate and lamivudine) and 5 (23.8%) receiving DTG (with tenofovir disoproxil fumarate and lamivudine), shown in Supplementary Table 1. The median $CD4^+$ T-cell count at ART initiation was 358/μL (interquartile range [IQR], 278–477/μL), with no difference between ART groups (Supplementary Table 2). The median viral load at ART initiation was 4.54 $\log_{10}$ RNA copies/mL (IQR, 3.85–4.80) in the EFV group versus 3.83 $\log_{10}$ RNA copies/mL (3.49–3.83) in the DTG group, with a combined viremia of 4.28 $\log_{10}$ RNA copies/mL (Supplementary Table 2). Both ART groups were on treatment for a median of 84 days (IQR, 44–105 days) and women in the DTG arm achieved viral suppression (cutoff ≤50 copies/mL or 1.69 $\log_{10}$ RNA copies/mL) at a faster rate, at 4 versus 2 weeks after delivery (Supplementary Table 2 and [18]). For the purposes of this study, we combined the placentas from 2 ART groups, because only 5 were collected from the DTG arm.

Effect of HIV on Placental Weight

Table 2 shows placenta characteristics and pathologic conditions stratified by HIV status. PWNH had significantly larger placentas (median weight [IQR], 468 [426–533] g) than PWH (394 [343–469] g; $P = .02$), with 38% of placentas in PWH having weight below the 10th percentile for gestational age, compared with 0% in PWNH. These differences were also reflected the in the fetal-placental ratios; all fetal-placental ratios above the 90th percentile were in the PWH ($P = .02$). Placental histopathology identified 2 cases (9.5%) of chronic deciduitis and 6 (28.6%) of maternal vascular malperfusion (MVM), all in PWH. There were no significant differences between PWH and PWNH in the incidence of meconium exposure and chorioamnionitis, and there was no evidence of villitis of unknown cause in any of the placentas. There were no significant differences in placental weight, fetal-placental ratio, or placental pathologic conditions between the 2 ART groups (Supplementary Table 3).

Effects of HIV Infection During Pregnancy on Placental $CD4^+$ and $CD8^+$ T-Cell Proportions

Figure 1A shows significantly lower proportions of $CD4^+$ T cells in decidua parietalis and basalis, but not in the villous tissue, comparing placentas from PWH with those from PWNH. The proportion of $CD8^+$ T cells was significantly increased (Figure

Table 1. Maternal and Infant Characteristics

Characteristic	PWNH (n = 9)	PWH (n = 21)	*P* Value
Maternal characteristics			
Age group, no. (%)			>.99
≤24 y	1 (11.1)	2 (9.5)	
25–29 y	5 (55.6)	11 (52.4)	
≥30 y	3 (33.3)	8 (38.1)	
Age, median (IQR), y	29 (25–31)	29 (26–30)	…
Gestation at enrollment, median (IQR)	30 (26–32)	28 (28–31)	.6
Gravidity, no. (%)			.003
1	5 (55.6)	1 (4.8)	
2	3 (33.3)	7 (33.3)	
≥3	1 (11.1)	13 (61.9)	
Gravidity, median (IQR)	1 (1–2)	3 (2–3)	…
Infant characteristics			
Sex, no. (%)			>.99
Female	6 (66.7)	12 (57.1)	
Male	3 (33.3)	6 (28.6)	
Data missing	0 (0)	3 (14.3)	
Gestation at delivery, median (IQR), completed wk	40 (40)	39 (38–39)	.003
Birth weight, median (IQR), g	3420 (3420–4000)	3305 (3010–3570)	.07

Abbreviations: IQR, interquartile range; PWH, pregnant women living with human immunodeficiency virus (HIV); PWNH, pregnant women not living with HIV.

Table 2. Placental Characteristics and Pathologic Conditions at Delivery

Characteristics and Pathologic Conditions	Placentas, No. (%)[a]		*P* Value
	From PWNH (n = 9)	From PWH (n = 21)	
Placental basal plate weight (g), median (IQR)	468 (426–533)	394 (343–469)	.02
Placental weight			
Small (<10th percentile)	0 (0)	8 (38.1)	.03
Appropriate (10th–90th percentile)	9 (100.0)	12 (57.1)	
Large (>90th percentile)	0 (0)	0 (0)	
Data missing	0 (0)	1 (4.8)	
Fetal-placental weight ratio			
Small (<10th percentile)	1 (11.1)	0 (0)	.02
Appropriate (10th–90th percentile)	8 (88.9)	11 (52.4)	
Large (>90th percentile)	0 (0)	7 (33.3)	
Data missing	0 (0)	3 (14.9)	
Placenta dimension, median (IQR)			
Greatest diameter, mm	180 (172–210)	180 (170–199)	.6
Thickness, mm	20 (15–20)	25 (16.5–27.5)	.2
Cord insertion			
Central	3 (33.3)	3 (33.3)	.7
Off center	5 (55.6)	12 (57.1)	
Marginal	1 (11.1)	5 (23.8)	
Data missing	0 (0)	1 (4.76)	
Pathologic conditions at delivery			
Meconium exposure			
Yes	3 (33.3)	2 (9.5)	.1
No	6 (66.7)	19 (90.5)	
Chorioamnionitis			
Maternal inflammatory response			
Yes	2 (22.2)	2 (9.5)	.6
No	7 (77.8)	19 (90.5)	
Fetal inflammatory response			
Yes	2 (22.2)	3 (14.3)	.6
No	7 (77.8)	18 (85.7)	
Chronic deciduitis			
Yes	0 (0)	2 (9.5)	.5
No	9 (100)	19 (90.5)	
Maternal vascular malperfusion			
Yes	0 (0)	6 (28.6)	.09
No	9 (100)	15 (71.4)	
Villitis of unknown cause			
Yes	0 (0)	0 (0)	>.99
No	9 (100)	21 (100)	

Abbreviations: IQR, interquartile range; PWH, pregnant women living with human immunodeficiency virus (HIV); PWNH, pregnant women not living with HIV.

[a]Data represent no. (%) of placentas unless other specified.

1A) in all 3 placental compartments, resulting in significantly lower CD4/CD8 T-cell ratios in the 3 placental tissues from PWH (Figure 1B). Notably, the inverted CD4/CD8 ratio in villous tissue was due to the increased $CD8^+$ T cells in the villous tissue. The inverted CD4/CD8 ratio was partially reflected in cord blood from HEU infants (Figure 1B). No differences were identified in T-cell proportions when stratified by the different ART regimens (Supplementary Figure 3). The proportions of $CD4^+$ and $CD8^+$ T cells in the placenta were not associated with placental weight, gravidity, or gestational age at delivery (Supplementary Figures 4, 5, and 6).

Maternal absolute peripheral blood $CD4^+$ T-cell counts, measured before ART at a median of 28 weeks' gestation, were positively correlated with the proportion of $CD4^+$ T cells in the decidua and negatively correlated with the proportion of $CD8^+$ T cells in decidua and villous tissue (Figure 1C and 1D). A similar trend was observed in cord blood. Thus, the inverted placental tissue CD4/CD8 T-cell ratios appeared to reflect the maternal peripheral immune status but were mirrored to a lesser extent in the cord blood of HEU infants. This correlation was temporally dissociated, where correlations were made between blood measured at 28 weeks and placentas measured at

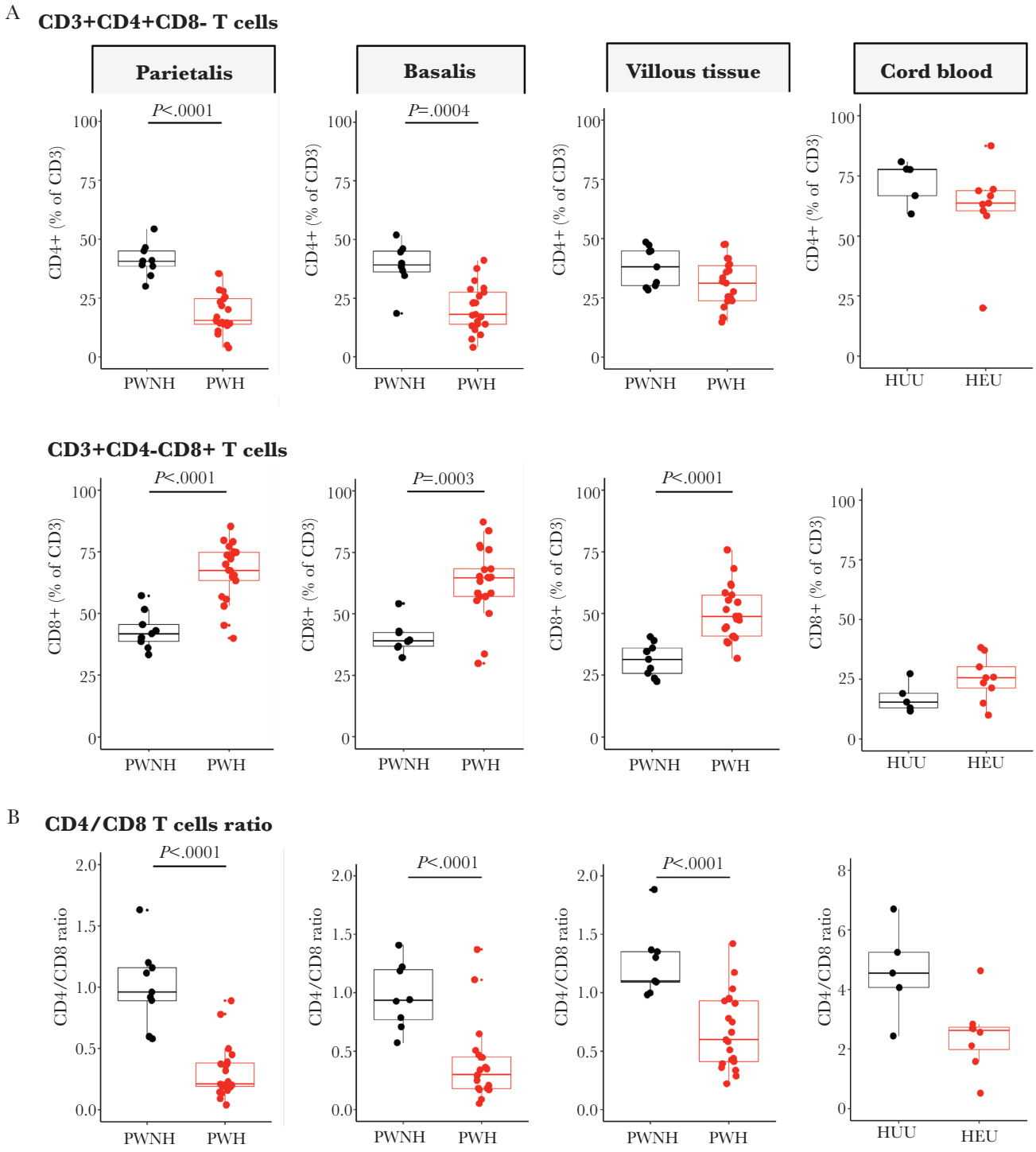


Figure 1. Proportions of T cells in the placenta and cord blood. A, Box plots (showing medians and interquartile ranges) of $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$ T-cell proportions isolated from the decidua parietalis, decidua basalis, villous tissue, and cord blood from pregnant women not living with human immunodeficiency virus (HIV) (PWNH) and pregnant women living with HIV (PWH) and cord blood from HIV-unexposed uninfected (HUU) and HIV exposed uninfected (HEU) infants. *B,* Box plots (showing medians and interquartile ranges) of CD4/CD8 T-cell ratios in the decidua parietalis, decidua basalis, villous tissue, and cord blood from PWNH and PWH and cord blood from HUU and HEU infants. Tests of significance were performed using the Mann-Whitney *U* test. *C,* Correlation plots between the absolute maternal $CD4^+$ T-cell count at 28 weeks' gestation before antiretroviral therapy (ART) initiation and the proportions of $CD4^+$ T cells isolated from the decidua parietalis, basalis, villous tissue, and cord blood. Statistical analysis was performed using the Spearman rank test; gray-shaded areas represent 95% confidence intervals. *D,* Correlation plots between the absolute maternal $CD4^+$ T-cell count at 28 weeks' gestation (before ART initiation) and the proportion of $CD8^+$ T cells isolated from the decidua parietalis, basalis, villous tissue. and cord blood. Statistical analysis was performed using the Spearman rank test, and gray-shaded areas represent 95% confidence intervals.

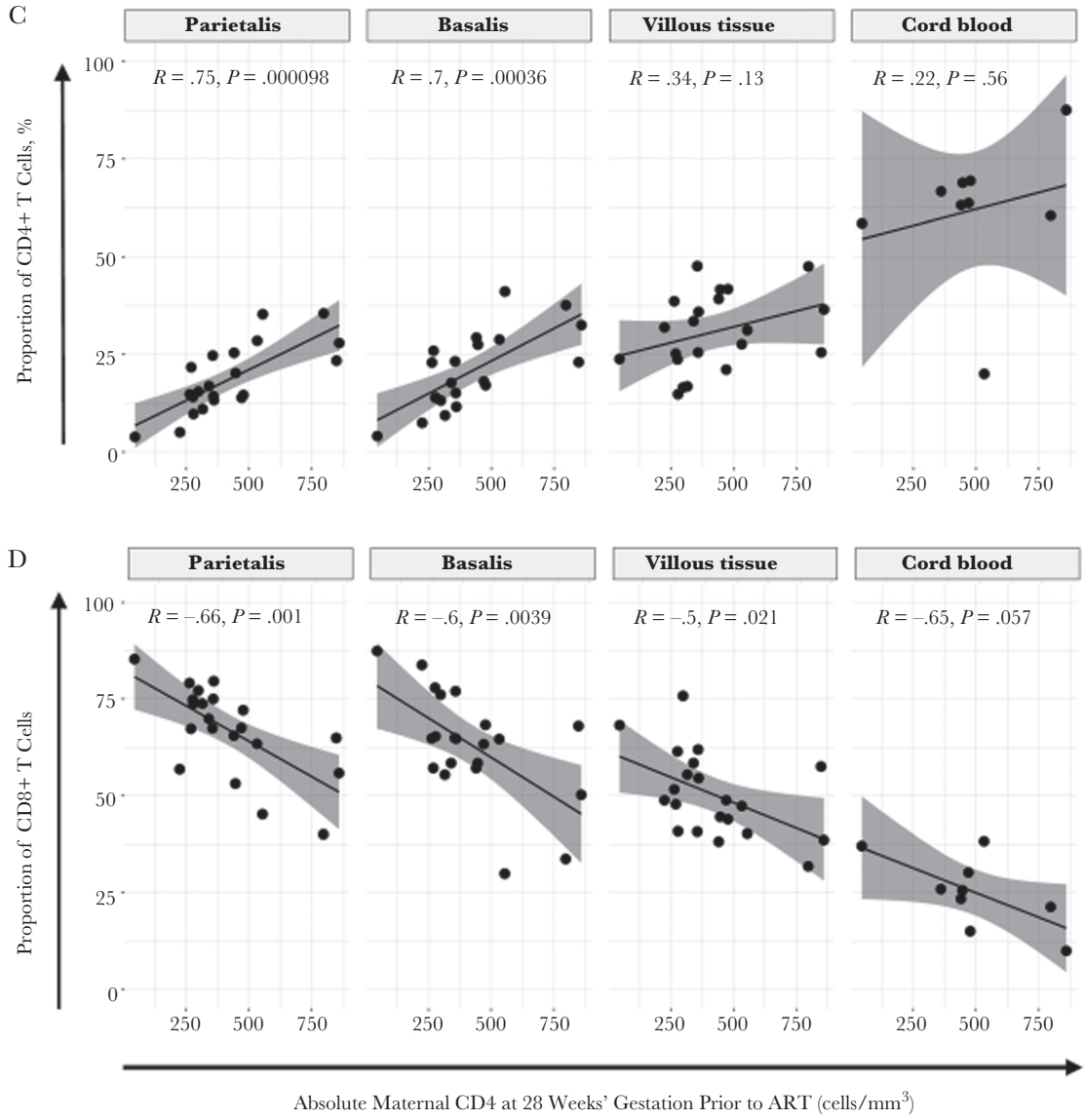


Figure 1. Continued.

38–40 weeks of gestation, suggesting that inverted T-cell ratios persisted throughout pregnancy.

Pre-ART Maternal Viral Load and Placental and Cord Blood CD4+ and CD8+ T-Cell Proportions

Maternal viremia dropped over time from enrollment to delivery (12 weeks beforehand), where PWH receiving DTG decreased at a faster rate (Figure 2A) [18]. As expected, the enrollment plasma viremia, ranging from 1.69–6.0 $\log_{10}$ RNA copies/mL, was significantly inversely correlated with the absolute maternal peripheral blood $CD4^+$ T-cell count determined before ART, at enrollment (Figure 2B). Pre-ART viremia also showed a significant negative correlation with proportions of $CD4^+$ T cells in the decidua parietalis and basalis (Figure 2C) and a positive correlation with $CD8^+$ T cells in the decidua parietalis, basalis and villous tissue at delivery (Figure 2D). The association between maternal viral load over time and the proportions of decidual $CD4^+$ and $CD8^+$ T cells was maintained at 8 and 4 weeks before delivery for $CD4^+$ T cells (Supplementary Figure 7) and up to 8 weeks before delivery for $CD8^+$ T cells (Supplementary Figure 8). Maternal viremia was not correlated with the proportion of T cells in the cord blood (Figure 2C and 2D), suggesting that maternal viremia (before and after ART initiation) can influence the homeostatic balance of T cells in the placenta, but not in the "newborn" immune compartment.

Immunohistochemical Confirmation of Anatomic Location and Fetal Origin of CD8+ T Cells in Villous Tissue

Analysis of placental villi from 13 PWH and 3 PWNH controls showed the presence of $CD8^+$ T cells in the fetal capillaries

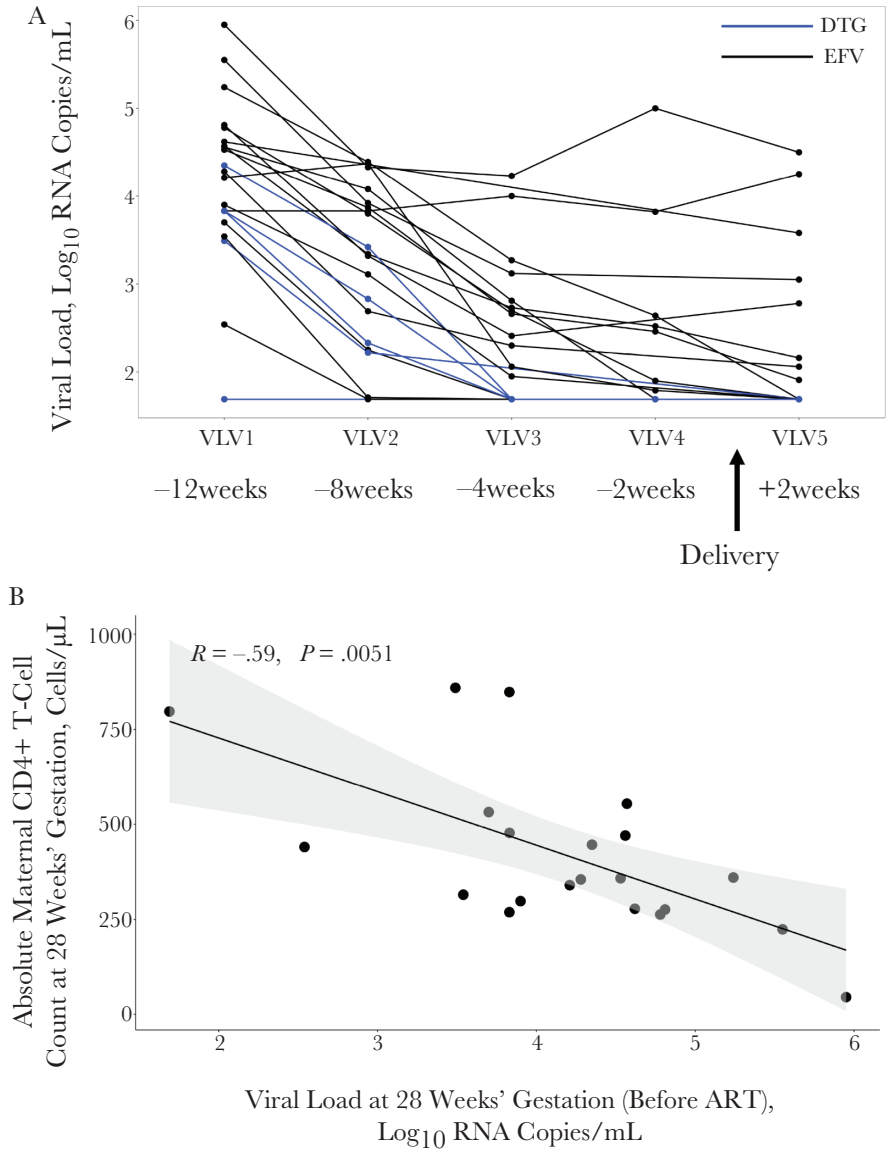
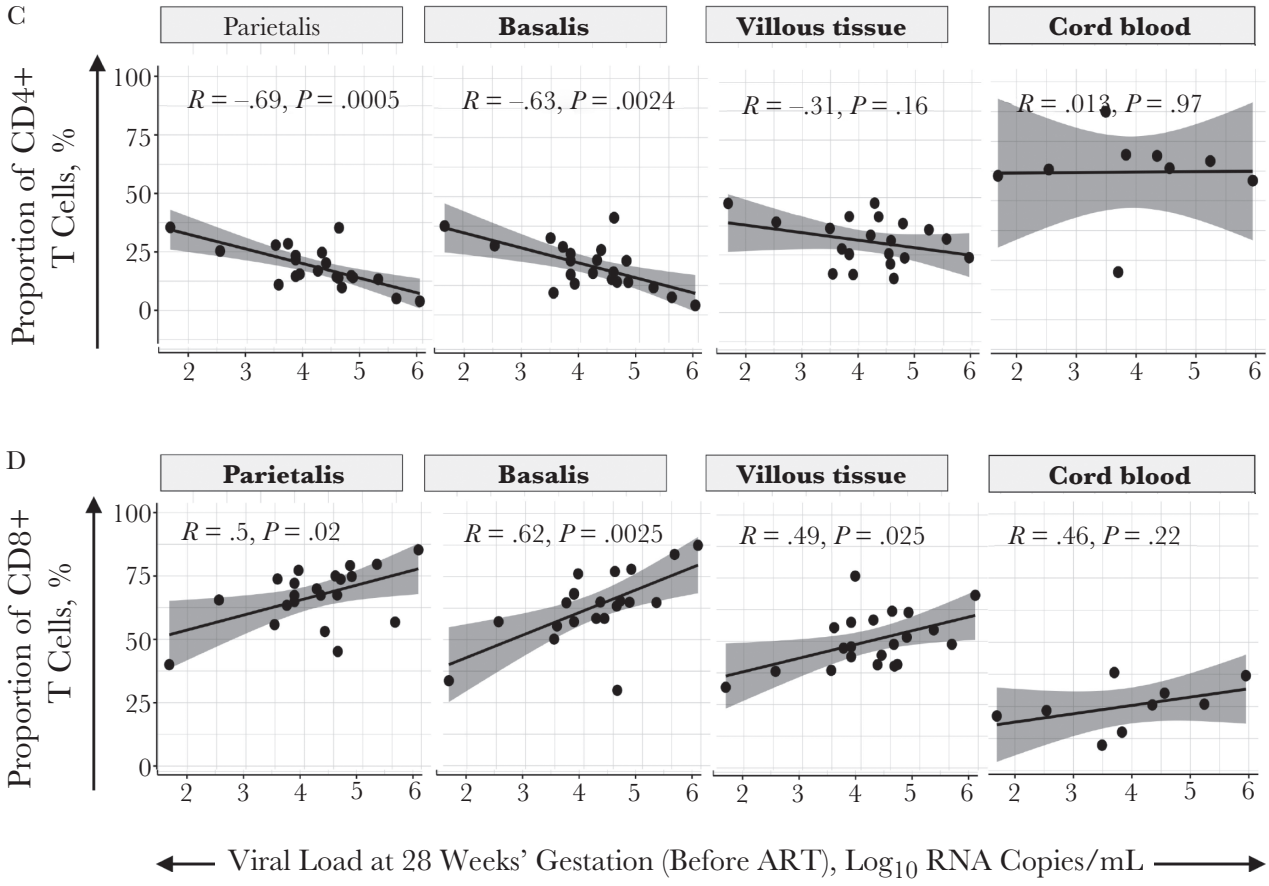


Figure 2. Proportions of $CD4^+$ and $CD8^+$ T cells in the placenta and maternal viral load. *A,* Line plot depicting participant viral load trajectories over time at antiretroviral therapy (ART) initiation, was at 28 weeks' gestation (VLV1; 12 weeks before delivery); at 29, 33, and 36 weeks' gestation (VLV2, VLV3, and VLV4, respectively; 8, 4, and 2 weeks before delivery); and at day 14 after delivery (VLV5; approximately 2 weeks after delivery). The women received efavirenz (EFV; with tenofovir disoproxil fumarate [TDF] and lamivudine [3TC]) (*black lines*) or dolutegravir (with TDF+3TC) (*blue lines*). *B,* Correlation plot between maternal systemic absolute $CD4^+$ T-cell counts at enrollment, before ART initiation, with maternal viral load at enrollment and ART initiation (at 28 weeks' gestation). Statistical analysis was performed using the Spearman rank test. *C,* Correlation plots between $CD4^+$ T-cell proportions in the placenta and maternal viral load at enrollment and ART initiation (28 weeks' gestation) in the decidua parietalis, basalis, villous tissue and cord blood. Statistical analysis was performed using the Spearman rank test, and gray-shaded areas represent 95% confidence intervals. *D,* Correlation plots between $CD8^+$ T-cell proportions in the placenta and maternal viral load at enrollment and ART initiation (28 weeks' gestation) in the decidua parietalis, basalis, villous tissue and cord blood. Statistical analysis was performed using the Spearman rank test, and gray-shaded area represents 95% confidence intervals.

(Figure 3A). The numbers of $CD8^+$ T cells were positively correlated with pre-ART maternal viremia (Figure 3B), confirming the flow cytometric analysis (Figure 1A and 1D). Using fluorescence in situ hybridization to detect X and Y chromosomes in cells located within the villi from 5 placentas with male births, we confirmed the presence of male (fetal) cells in fetal capillaries (Figure 3C). Thus, the immunohistochemistry analysis confirms the anatomic location and fetal origin of the increased proportions of $CD8^+$ T cells in villous tissue. The fetal (male) origin of the $CD8^+$ T cells in placental villi is consistent with the absence of villitis of unknown cause, a lesion characterized by maternal immune infiltrates [23, 24].

HIV Exposure and Differentiation of $CD8^+$ T Cells in Placental Villi, Fetal Cord Blood, and Maternal Placental Compartments

CD45RA and CD28 were used to identify the proportions of naive ($CD45RA^+CD28^+$), early-differentiated ($CD45RA^-CD28^+$), late-differentiated ($CD45RA^-CD28^-$) and terminally



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differentiated ($CD45RA^+CD28^-$) memory $CD4^+$ and $CD8^+$ T cells from villous tissue and matching cord blood (Figure 4A). We observed significantly lower proportions of naive $CD8^+$ T cells and significantly higher proportions of late-differentiated $CD8^+$ T cells in villous tissue and cord blood of HEU compared with HUU infants (Figure 4B). The $CD4^+$ T cells in the villous tissue were predominantly of a naive and early-differentiated phenotype, while the cord blood cells were predominantly naive. There were no significant differences in $CD4^+$ T-cell differentiation state based on HIV exposure (Supplementary Figure 9). In addition, no significant differences in the stage of $CD4^+$ and $CD8^+$ T-cell differentiation in the decidua were observed between the HIV groups (Supplementary Figure 10). Thus, the increased differentiation state of $CD8^+$ T cells is confined to the fetal placental and cord blood compartments and not observed in the maternal placental compartments. There was no correlation between maternal pre-ART viremia and memory stage of $CD8^+$ T cells in placental villous tissue and cord blood (data not shown). We also showed that there was no association between villous tissue $CD8^+$ T-cell memory differentiation and reported adverse events in the infant during the first 12 weeks of life (Supplementary Figure 11).

DISCUSSION

We present data from a unique cohort of PWH who initiated ART late in pregnancy and show that maternal HIV infection has a clear impact on T-cell subsets in the decidua, villous tissue and cord blood. As the decidua and decidual immune cells are of maternal origin, it may not be surprising to find such a footprint [25]. Maternal HIV infection likely affects and kills maternal decidual $CD4^+$ T cells and fewer peripheral blood T cells may traffic to decidual tissue; chemokine gradients have been shown to play a key role in the trafficking of maternal T cells into the decidua during pregnancy [26]. We show that, in contrast to the decidua, the inverted CD4/CD8 ratio in the villous tissue was largely due to an increased proportion of $CD8^+$ T cells and not, as observed in the other tissues, due to a decrease in $CD4^+$ T cells. These $CD8^+$ T cells had an early to late memory differentiated phenotype, suggestive of previous antigen experience [27, 28].

The human placenta has 2 circulatory compartments: the uteroplacental unit for the trafficking of maternal blood and the fetoplacental unit for the fetal blood circulation [29, 30]. Therefore, cells in the villous tissue are likely to be predominantly from the placental reservoir. A key question is whether

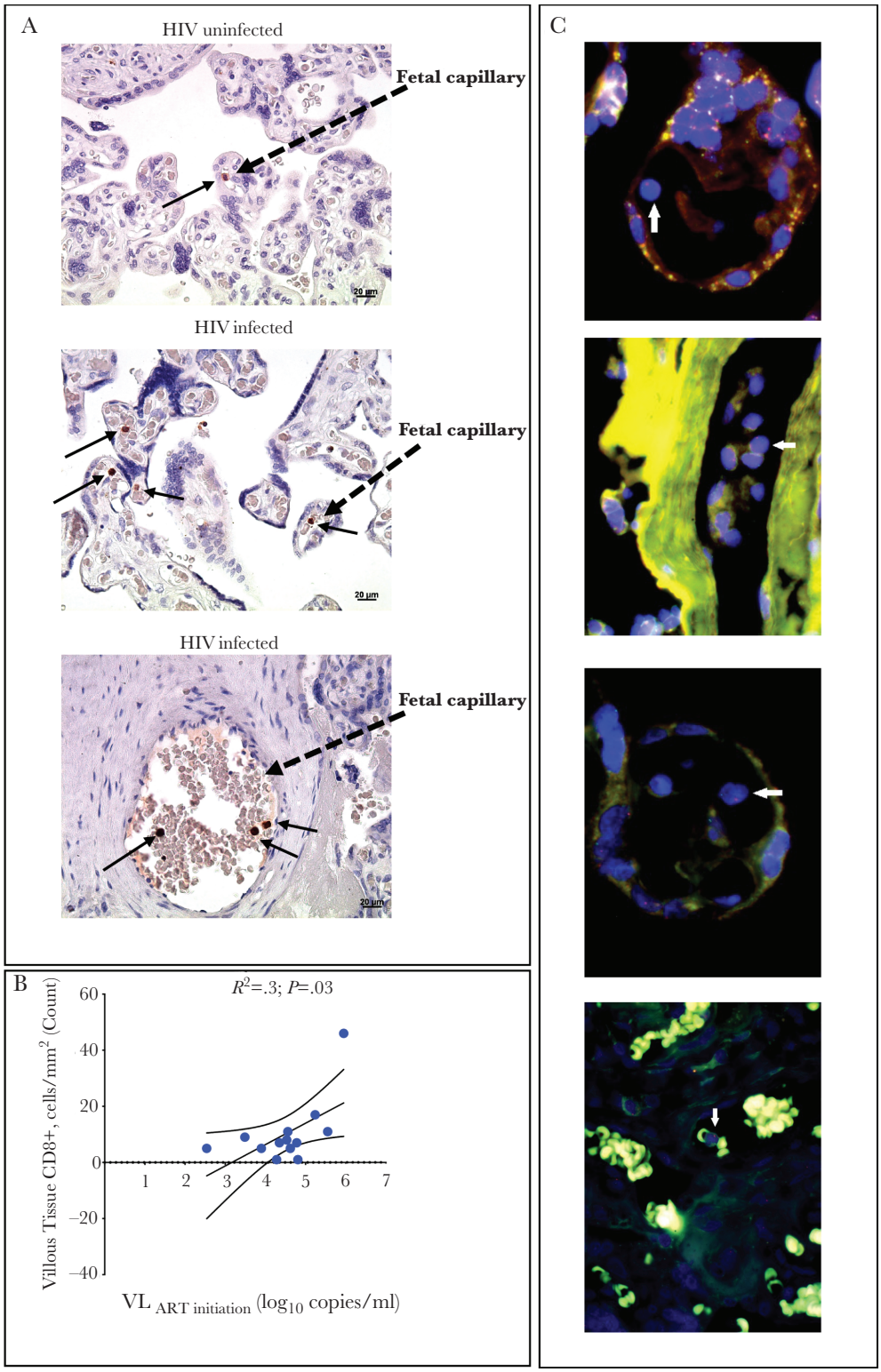

Figure 3. Anatomic location of CD8$^+$ T cells in the villous tissue. *A,* Representative immunohistochemical stained images of CD8$^+$ T cells in villous tissue sections denoted by brown dots and black arrows in the villi of placentas from human immunodeficiency virus (HIV)–infected and HIV-uninfected mothers (×40 magnification). *B,* Correlation plot between the density of tissue-bound CD8$^+$ T cells in the villi and maternal viral load (VL) at antiretroviral therapy (ART) initiation. Statistical analysis was performed using the Spearman rank test; curved lines represent the 95% confidence intervals. *C,* Representative fluorescence in situ hybridization images of lymphocytes (*arrows*) in the villous tissue from placentas from male infants. The X chromosome is denoted in green, and the Y chromosome in red (digitally scanned slides).

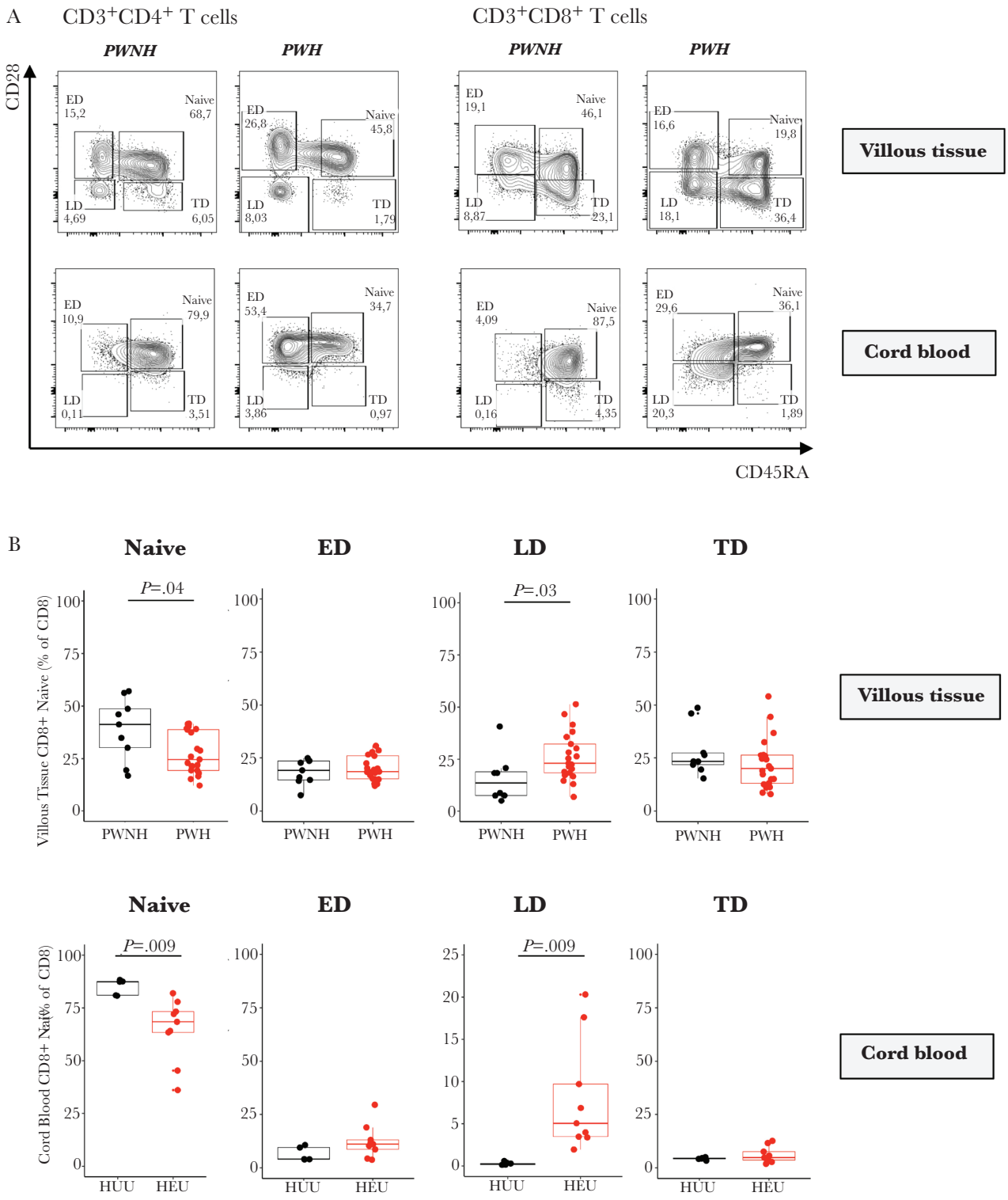

Figure 4. Memory phenotype of $CD4^+$ and $CD8^+$ T cells in the villous tissue. *A*, Representative flow cytometric contour plots of naive, early-differentiated (ED), late-differentiated (LD), and terminally differentiated (TD) $CD4^+$ and $CD8^+$ T cells in the villous tissue (of placentas from pregnant women not living with human immunodeficiency virus (HIV) (PWNH) and pregnant women living with HIV (PWH) and cord blood from HIV-unexposed uninfected (HUU) and HIV-exposed uninfected (HEU) infants. *B*, Box plots (showing medians and interquartile ranges) of $CD8^+$-naive, ED, LD, and TD T cells in the villous tissue and cord blood from HUU and HEU infants. Tests of significance were performed using the Mann-Whitney *U* test.

the increased $CD8^+$ T-cell differentiation in villi and cord blood is due to direct exposure to HIV antigens, presence of other pathogens (eg, cytomegalovirus) or increased levels of other noninfectious inflammatory cues in placentas of PWH. Viral particles and structural and core HIV proteins have been shown to cross the placental barrier in the absence of fetal infection, leading to altered immune profiles in HEU infants [31, 32]. A number of studies also describe HIV-specific T-cell responses in HEU infants, possibly primed by exposure to HIV antigens in utero [16, 33, 34].

The lower proportions of naive cells and increased memory T cells reflected in villous tissue and cord blood mirror findings of previous studies. HEU infants have been shown to have reduced $CD4^+$ and increased $CD8^+$ T-cell counts compared with HUU infants at birth [35, 36]; they have also been shown to have lower numbers of naive cells, a finding thought to be due to thymic involution and frequent stimulation and expansion of the antigen-specific T cells in an effort to regenerate the T-cell pool [14, 15]. Whether the same findings would be recapitulated in PWH with suppressed viral loads before conception is unknown. Nevertheless, this study afforded us a unique opportunity to investigate the impact of starting ART late in pregnancy and how this affected placental immunity. Comparing these findings with those in women who are consistently receiving ART throughout pregnancy would reveal whether long-term viral suppression creates a more normalized immune balance in the placenta.

It was not possible to tease out and distinguish the effects of HIV and ART on the placenta and fetus. There is evidence that some ART can cross the placenta [37, 38]. Additional studies show that perinatal drug exposure is associated with lower T-cell counts in the first 2 years of life in HEU infants [39]. Although we cannot discount the effect of ART alone on the placenta, the parent study did report minimal effects of ART on newborns [18]. One limitation of our study was that we had maternal $CD4^+$ and $CD8^+$ T-cell counts at 28 weeks' gestation only in PWH, with no equivalent measures in PWNH. We therefore could not directly compare the effects of HIV/ART exposure on systemic T cells with those to placental T cells at delivery, or with findings in healthy mothers.

The elevated proportions of $CD8^+$ T cells found embedded in the villi from PWH were shown to be sequestered within the fetal capillaries. These $CD8^+$ T cells within villi were proportional to maternal viremia. The absence of overt villitis of unknown cause corroborates the finding that expanded $CD8^+$ T-cell fractions are of fetal and not maternal origin. Previous studies have suggested that the presence of T cells in the villi during normal pregnancy reflects villitis of unknown cause [24]. However, there is emerging evidence, using immunohistochemical staining of villi sections from early elective termination placentas, of the presence of $CD45^+$ αβ T cells, although these cells were undetectable at term [40]. In a separate study, in which there were no reported maternal infections, the villous tissue was shown to contain a mixture of fetal and maternal immune cells [41]. We cannot discount the possibility that there was also a mix of maternal and fetal $CD8^+$ T cells in placental villi in our study.

The current study findings demonstrate that, within the fetoplacental unit, there are differences between the T-cell profiles in the villi and cord blood. We postulate that the cells we characterized in the villi may be a combination of cells within the villi and cells attached to the fetal capillaries. Contaminating circulating cord blood cells in this fraction cannot be completely ruled out, but this possibility is minimized by extensive washing of the tissues prior to isolation. Moreover, the phenotypic differences between villous and cord blood T cells as presented here suggest that the villous T cells do not include large proportions of circulating T cells. The increased differentiation state of $CD8^+$ T cells in the villi may be due to exposure to antigens within the villous microenvironment, as well as the mechanisms of immune activation leading to T-cell adhesion and extravasation. No association was revealed by attempts to make a direct connection between the elevated numbers and proportions of $CD8^+$ T cells found in the villous tissue with adverse health events in the first few weeks after birth; most events were resolving rashes.

Of particular note, women were ART naive during the first and second trimesters, and it is likely that prolonged HIV exposure may have contributed to altered placental development and the significantly lower placental weight observed. Interestingly, all cases of MVM were reported in placentas from PWH and possibly reflect placental injury, affecting maternal vasculature and perfusion and increasing the risk of an adverse birth outcome[42]. We have previously reported on MVM in placentas from PWH on long-term ART [21], an incidence of about 27% overall, similar to that reported by Kalk et al [6]. It is likely that HIV and/or ART exposure alters factors involved in vascular development, resulting in placental insufficiency and increased risk of adverse birth outcomes [8, 9, 43].

In conclusion, we provide evidence that in utero exposure to HIV results in an altered immune balance in both the uteroplacental and fetoplacental compartments. Despite the initiation of ART in the third trimester, resulting in either full or partial maternal viral suppression by the time of delivery, there was a significant imbalance in term placental T-cell homeostasis, and to a lesser degree in the cord blood. Overall, our results suggest that maternal immunity leaves a footprint in the placenta that may shape the neonatal/infant immune landscape.

Supplementary Data

Supplementary materials are available at *The Journal of 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.

Notes

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