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Higher CCR5 density on CD4$^+$ T-cells in mothers and infants is associated with increased risk of in-utero HIV-1 transmission

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Objective: CCR5-tropic viruses are preferentially transmitted during perinatal HIV-1 infection. CCR5 density on CD4$^+$ T-cells likely impacts susceptibility to HIV-1 infection.

Design: Fifty-two mother–infant dyads were enrolled. All mothers were living with HIV-1, 27 of the infants acquired HIV-1 *in utero* and 25 infants remained uninfected.

Methods: CCR5 density, together with frequencies of CD4$^+$ and CD8$^+$ T-cells expressing immune activation (CCR5, ICOS and HLA-DR) and immune checkpoint (TIGIT and PD-1) markers, were measured in whole blood from the dyads close to delivery.

Results: Compared with mothers who did not transmit, mothers who transmitted HIV-1 had less exposure to ART during pregnancy ($P = 0.015$) and higher plasma viral load close to delivery ($P = 0.0005$). These mothers, additionally, had higher CCR5 density on CD4$^+$ and CD8$^+$ T-cells and higher frequencies of CCR5, ICOS and TIGIT-expressing CD8$^+$ T-cells. Similarly, compared with infants without HIV-1, infants with HIV-1 had higher CCR5 density on CD4$^+$ and CD8$^+$ T-cells and higher frequencies of CCR5, TIGIT, and PD-1-expressing CD4$^+$ and CD8$^+$ T-cells as well as higher frequencies of HLA-DR-expressing CD8$^+$ T-cells. CCR5 density on maternal CD4$^+$ T-cells remained significantly associated with transmission after adjusting for maternal viral load and CD4$^+$ T cell counts. Mother–infant dyads with shared high CCR5 density phenotypes had the highest risk of transmission/acquisition of infection compared with dyads with shared low-CCR5 density phenotypes.

Conclusion: This study provides strong evidence of a protective role for a combined mother–infant low CD4$^+$ T-cell CCR5 density phenotype in in-utero transmission/acquisition of HIV-1.

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Keywords: CCR5 density, CD4$^+$ T cell, HIV-1 transmission, *in utero*, mothers and infants

Introduction

CCR5 is the major coreceptor for HIV-1 entry [1–5] and is critical for vertical transmission of HIV-1 as CCR5-tropic viruses are preferentially transmitted from mothers to their infants [6,7]. CCR5Δ32 homozygosity in infants confers protection from vertical transmission [8,9]. CCR5Δ32 heterozygosity, however, is associated with reduced risk of vertical HIV-1-infection in some studies [8,10] but not in others [11]. CCR5 expression associates with the susceptibility of cells to HIV-1 infection [12,13]. CCR5 density on the surface of CD4$^+$ T-cells is lower in

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HIV-1-exposed uninfected individuals compared with HIV-1-unexposed individuals [14,15], and in HIV-1 controllers compared with noncontrollers [16,17].

HIV-1 preferentially infects activated $CD4^+$ T-cells [18]. Thus, individuals with higher frequencies of activated $CD4^+$ T-cells, may have increased risk of infection because of the increased availability of activated target cells. In individuals exposed to HIV-1, lower levels of immune activation are associated with a decreased risk of HIV-1 acquisition [19]. T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) and programmed cell death protein-1 (PD-1 or CD279) are immune checkpoint receptors, which are upregulated upon activation of T-cells to prevent excessive immune activation [20]. Overexpression of immune checkpoint receptors occurs during HIV-1 infection and is associated with T-cell exhaustion and dysfunction [21–23]. Their expression correlates with HIV-1 disease progression [24–26] similarly to markers of immune activation [27]. Additionally, frequencies of $CD8^+$ T-cells expressing the immune activation marker, CD38, positively correlate with frequencies of $CD8^+$ T-cells expressing the immune checkpoint molecule, PD-1, in people with HIV-1 (PWH) [24,26]. Although immune activation and immune checkpoint molecules have contrasting functions, they correlate positively with HIV-1 disease progression and with each other. Aside from being an HIV-1 coreceptor, there is evidence supporting a role for CCR5 in T-cell activation [28,29]. Furthermore, CCR5 density on $CD4^+$ T-cells correlates with frequencies of activated $CD38^+$ $CD8^+$ T-cells, independently of viral load, in PWH [30]. Although antiretroviral therapy (ART) initiation decreases cellular activation, it does not significantly impact HLA-DR-CD4+ T-cell CCR5 density [30]. CCR5 density on nonactivated $CD4^+$ T-cells is stable over time in adults [17] and in children [31] living with HIV-1.

Given the crucial role of CCR5 in HIV-1 acquisition and disease progression, we hypothesized that CCR5 density on $CD4^+$ T-cells associates with HIV-1 vertical transmission, and that a combined higher maternal–infant $CD4^+$ T-cell CCR5 density would associate with a higher risk of in-utero infection. To address this, we analysed CCR5 expression (density and frequency) on $CD4^+$ and $CD8^+$ T-cells, as well as frequencies of $CD4^+$ and $CD8^+$ T-cells expressing immune activation [CCR5, inducible costimulator (ICOS) and HLA-DR] and immune checkpoint molecules (TIGIT and PD-1), in mothers and their infants in the context of risk of in-utero HIV-1 transmission.

Materials and methods

Study participants

A total of 52 mother–infant dyads were enrolled into this study and were a subset of infants enrolled into the Latency and Early Neonatal Provision of Antiretroviral Drugs (LEOPARD) study at Rahima Moosa Mother and Child Hospital in Johannesburg, South Africa [32,33]. All 52 mothers were living with HIV-1. Of these, 27 transmitted HIV-1 to their infants [transmitting mothers) and 25 did not [nontransmitting mothers (NTMs)]. Infants with HIV-1 tested positive and infants without HIV-1 (HIV-exposed uninfected: HEU) tested negative on a diagnostic HIV-1 total nucleic acid (TNA) PCR on a sample collected within 48 h of birth. Enrollment samples were collected between less than 48 h and 19 days after birth (Supplemental Table 1, http://links.lww.com/QAD/D121). Samples collected between 31 August 2015 and 1 February 2017 were analysed.

This study was reviewed and approved by Human Research Ethics Committee (HREC) of the University of the Witwatersrand and the Institutional Review Board (IRB) of Columbia University. Written informed consent to participate in the study was provided by the participants' legal guardian/next of kin.

Clinical covariates

Viral load for the mothers and infants with HIV-1 was measured using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, USA) with a limit of detection of 20 copies/ml. $CD4^+$ T-cell counts were measured using the TruCOUNT method (BD Biosciences, San Jose, USA). The maternal plasma viral loads and $CD4^+$ T-cell counts were measured soon after delivery. Infant viral loads, $CD4^+$ T-cell counts and percentages were measured at baseline before treatment.

Maternal (demographic characteristics, syphilis serology, HIV diagnosis, ART during pregnancy and breastfeeding) and infant data (mode of delivery, gestational age, birth weight, sex and antiretroviral prophylaxis) were collected.

Flow cytometry

EDTA-anticoagulated whole blood samples were stained within 6 h of collection. Briefly, 100 μl of whole blood was stained with an antibody cocktail, diluted in Brilliant Stain Buffer (BD Biosciences). Red blood cells were lysed with FACS lysing solution (BD Biosciences), samples washed and resuspended in FACSflow. Two different antibody panels were used. One panel contained CD8 PerCP (SK1), CD4 FITC (SK3), CD3 APC-H7 (SK7), PD-1 BV786 (EH12.1) and ICOS BV650 (DX29) from BD Biosciences and the other panel contained CD8 Alexa Fluor 700 (RPA-T8), CD4 BV786 (L200), CD3 APC-H7 (SK7), TIGIT APC (MBSA43), CCR5 PE (2D7) from BD Biosciences and HLA-DR PE-Cy5.5 (TU36) from Invitrogen (Waltham, USA). CCR5 density was calculated using BD Quantibrite™ beads (BD Biosciences) [34]. Samples were acquired on a four laser BD LSRFortessa X-20 (BD Biosciences) within 4 h.

CS&T beads and mid-range Rainbow Fluorescent Particles (BD Biosciences) were run before sample acquisition. Compensation was performed for each experiment using BD CompBeads (BD Biosciences). Samples were analyzed using FlowJo software version 9.9.6 (BD Biosciences).

The gating strategy is shown in Supplemental Figure 1A, http://links.lww.com/QAD/D121. A representative example of expression of these markers in a mother–infant pair is shown in Supplemental Figure 1B, http://links.lww.com/QAD/D121.

Statistical analyses

Baseline clinical characteristics of the transmitting mothers vs. NTMs and the infants with HIV-1 (HIV) vs. HEU were compared using the Fisher's exact test for categorical variables and the Mann–Whitney *U* non-parametric test for continuous variables.

CCR5 density and the frequency of $CD4^+$ and $CD8^+$ T-cells expressing immune activation and immune checkpoint markers between mothers and their infants were compared using the Wilcoxon matched-pairs signed rank test. The Mann–Whitney *U* test was used to compare these parameters between transmitting mothers vs. NTMs and infants with HIV-1 vs. HEU infants. Spearman correlation coefficients were used to analyse correlations between maternal and infant cell subsets and markers of HIV-1 disease severity ($CD4^+$ T-cell count, $CD4^+$ T-cell percentage and HIV-1 viral load). The Fisher's exact test was used to compare proportions of NTM/HEU and TM/HIV dyads with high and low CCR5 density on $CD4^+$ T-cells.

Variables associated with perinatal transmission by Mann–Whitney *U* test were used in logistic regression models. Univariate logistic regression analyses evaluated predictive associations between cell subsets and risk of HIV-1 transmission. Multivariate logistic regression was used to adjust for maternal viral load and $CD4^+$ T-cell counts. Statistical analyses were performed using STATA version 12.1 (StataCorp. 2011, College Station, USA). GraphPad Prism 5 (GraphPad Software, San Diego, USA) and STATA were used for graphical presentations.

Results

Characteristics of the study population

Characteristics of the mother–infant pairs are presented in Supplemental Table 1, http://links.lww.com/QAD/D121. Maternal age, education, parity, mode of delivery, breastfeeding, syphilis serology, timing of HIV-1 diagnosis, maternal $CD4^+$ T-cell count and $CD4^+$: $CD8^+$ ratio and length of gestation did not differ significantly between mothers who did and did not transmit. All mothers who did not transmit and 81% of the mothers who transmitted received ART (efavirenz, emtricitabine and tenofovir disoproxil fumarate) during pregnancy, with ART initiation occurring earlier in mothers who did not transmit. Maternal viral loads close to delivery were higher among mothers who transmitted compared with those who did not. Infant birth weight and sex did not differ between infants with and without HIV-1. All of the uninfected infants and 25 of the 27 infants with HIV-1 received nevirapine prophylaxis. Infant $CD4^+$: $CD8^+$ ratio at enrollment did not differ significantly between infants with and without HIV-1.

Comparison of mothers who did or did not transmit

CCR5 density on $CD4^+$ and $CD8^+$ T-cells and the frequency of $CD4^+$ and $CD8^+$ T-cells expressing immune activation (CCR5, ICOS, and HLA-DR) and immune checkpoint receptors (TIGIT and PD-1) was compared between mothers who did and did not transmit. Mothers who transmitted had higher CCR5 density on both $CD4^+$ and $CD8^+$ T-cells and higher frequencies of CCR5+, ICOS+ and TIGIT+ $CD8^+$ T-cells than mothers who did not transmit (Fig. 1).

All maternal T-cell subsets that were significantly different between the two groups correlated positively with viral load and negatively with $CD4^+$ T-cell count in the total group of mothers – with the exception of CCR5 density on $CD4^+$ T-cells, which did not correlate with $CD4^+$ T-cell count (Supplemental Figure 2, http://links.lww.com/QAD/D121). After adjustment for maternal viral load and $CD4^+$ T-cell count, CCR5 density on $CD4^+$ T-cells and the frequency of ICOS+$CD8^+$ T-cells remained significantly associated with increased risk for transmission (Table 1 – model 1). When we evaluated which of the maternal subsets were associated with transmission taking into account the five variables together (data not shown) – CCR5 density on $CD4^+$ and $CD8^+$ T-cells and the frequency of ICOS+$CD8^+$ T-cell subsets were each independently associated with transmission and remained so after adjusting for viral load and $CD4^+$ T-cell count. These three variables were then analysed together (Table 1 – model 2), following which all were significant (unadjusted and when adjusted for viral load and $CD4^+$ T-cell count), but in contrast to model 1, CCR5 density on $CD8^+$ T-cells associated with reduced vertical transmission.

Comparison of infants who did or did not acquire HIV-1

Compared with infants without HIV-1, infants with HIV-1 had a higher CCR5 density on $CD4^+$ and $CD8^+$ T-cells, higher frequencies of CCR5+, TIGIT+, PD-1+ CD4+ and $CD8^+$ T-cells as well as higher frequencies of HLA-DR+ $CD8^+$ T-cells (Fig. 1).

After adjusting for maternal viral load and maternal $CD4^+$ T-cell count, CCR5 density on $CD4^+$ T and

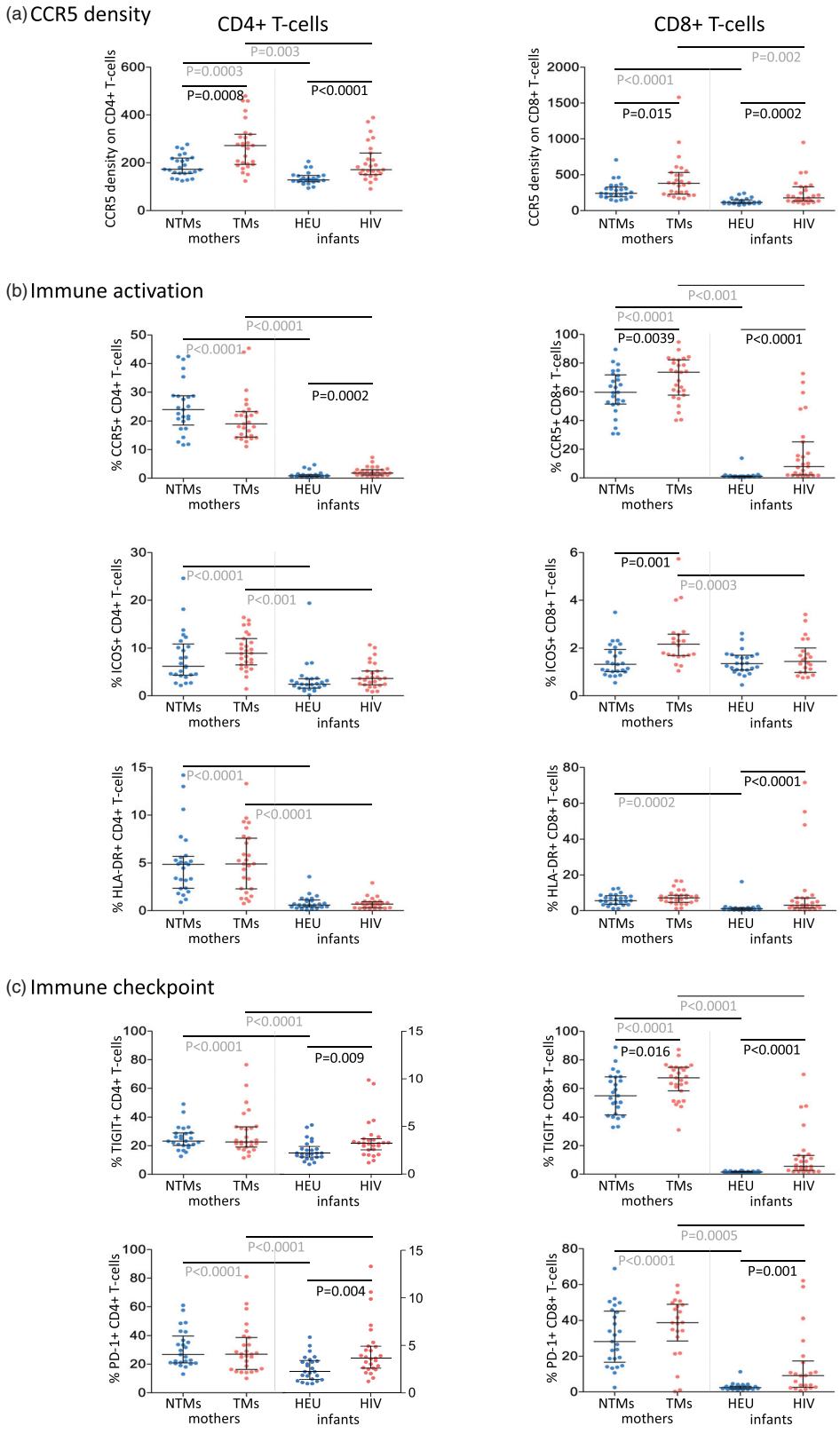

Fig. 1. Expression of CCR5 density, immune activation and immune checkpoint markers. $CD4^+$ and $CD8^+$ T-cells in non-transmitting (NTMs) and transmitting mothers (TMs) and their infants were stained for the analysis of (a) CCR5 density, (b) immune activation and (c) immune checkpoint markers. Each symbol represents an individual. Horizontal lines and error bars represent the median, 25th and 75th percentiles. Significant differences between NTMs and transmitting mothers, and between HIV-exposed uninfected (HEU) infants and infants with HIV-1 (HIV) are shown in black and between mothers and their infants are shown in grey.

Table 1. Logistic regression results predicting transmitter status from maternal subsets and acquisition of infection from infant subsets unadjusted and adjusted for maternal viral load and CD4$^+$ T-cell counts.

	Unadjusted			Adjusted for maternal VL			Adjusted for maternal CD4$^+$ T-cell counts			Adjusted for maternal VL and CD4$^+$ T-cell counts		
Subset	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Maternal subsets	Model 1[a]											
CCR5 density on CD4$^+$ T-cells	1.02	1.01–1.03	**0.003**	1.01	1.0–1.02	**0.012**	1.02	1.01–1.03	**0.003**	1.01	1.00–1.03	**0.011**
CCR5 density on CD8$^+$ T-cells	1.005	1.00–1.01	**0.029**	1	1.00–1.01	0.16	1.01	1.00–1.01	**0.037**	1.003	1.00–1.01	0.130
% CCR5+ CD8$^+$ T-cells	1.04	1.00–1.08	**0.043**	1.02	0.98–1.07	0.33	1.04	1.00–1.09	0.071	1.03	0.98–1.08	0.280
% ICOS+ CD8$^+$ T-cells	4.4	1.48–12.83	**0.004**	2.6	0.90–7.78	0.08	4.93	1.56–15.54	**0.006**	3.16	1.02–9.84	**0.046**
% TIGIT+ CD8$^+$ T-cells	1.05	1.01–1.10	**0.025**	1.02	0.96–1.07	0.56	1.05	1.00–1.10	**0.039**	1.02	0.96–1.07	0.540
	Model 2[b]											
CCR5 density on CD4$^+$ T-cells	1.06	1.02–1.10	**0.006**	1.06	1.02–1.11	**0.009**	1.06	1.01–1.10	**0.008**	1.06	1.01–1.11	**0.013**
CCR5 density on CD8$^+$ T-cells	0.98	0.96–1.00	**0.020**	0.97	0.95–1.00	**0.013**	0.98	0.96–1.00	**0.027**	0.97	0.95–1.00	**0.021**
% ICOS+ CD8$^+$ T-cells	9.87	2.06–47.25	**0.004**	7.26	1.46–36.06	**0.015**	9.85	2.02–48.13	**0.005**	7.74	1.51–39.58	**0.014**
Infant subsets												
CCR5 density on CD4$^+$ T-cells	1.04	1.01–1.06	**0.003**	1.03	1.01–1.06	**0.009**	1.04	1.01–1.07	**0.002**	1.04	1.01–1.06	**0.008**
CCR5 density on CD8$^+$ T-cells	1.02	1.00–1.03	**0.008**	1.02	1.00–1.03	**0.025**	1.02	1.01–1.03	**0.007**	1.02	1.0–1.03	**0.025**
% CCR5+ CD4$^+$ T-cells	2.11	1.17–3.83	**0.013**	1.92	1.05–3.50	**0.031**	2.11	1.16–3.84	**0.014**	1.92	1.06–3.5	**0.031**
% CCR5+ CD8$^+$ T-cells	Undefined[c]											
% HLA-DR+ CD8$^+$ T-cells	Undefined[c]											
% TIGIT+ CD4$^+$ T-cells	1.86	1.06–3.26	**0.031**	2.25	1.12–4.50	**0.022**	1.83	1.04–3.23	**0.037**	2.36	1.17–4.78	**0.017**
% TIGIT+ CD8$^+$ T-cells	Undefined[c]											
% PD-1+ CD4$^+$ T-cells	1.75	1.12–2.73	**0.014**	1.77	1.09–2.87	**0.021**	1.74	1.11–2.74	**0.016**	1.80	1.13–2.87	**0.014**
% PD-1+ CD8$^+$ T-cells	Undefined[c]											

Statistically significant results are shown in bold. CI, confidence interval.

[a]Model 1 examines maternal subsets (which were significantly different between mothers who did and did not transmit (Fig. 1), shown individually unadjusted (column 1), then adjusted for maternal viral load (column 2), maternal CD4$^+$ T-cell counts (column 3) and both maternal viral load and CD4$^+$ T cell counts simultaneously (column 4). These five subsets were combined in another model (i.e. takes into account all the subsets together), which after adjustment for both maternal viral load and CD4$^+$ T-cell counts identified the three subsets that associated significantly with transmission and were used in model 2.

[b]Model 2 includes three maternal subsets shown together unadjusted (column 1), then adjusted for maternal viral load (column 2), maternal CD4$^+$ T-cell counts (column 3) and both maternal viral load and CD4$^+$ T-cell counts (column 4).

[c]Completely distinguished between the groups.

$CD8^+$ T-cells, and frequencies of CCR5+, TIGIT+, and PD-1+ $CD4^+$ T-cells remained significantly associated with increased risk for acquisition of infection (Table 1). We could not undertake adjusted analyses for frequencies of CCR5+, HLA-DR+, TIGIT+, and PD-1+ $CD8^+$ T-cells as these markers were so strongly related to infection, they almost completely distinguished between the groups.

Of the infant T-cell subsets that were significantly different between infants with and without HIV-1, CCR5 density on $CD4^+$ T-cells, frequency of CCR5+ and TIGIT+ $CD8^+$ T-cells correlated positively with infant viral load and frequency of CCR5+ and TIGIT+ $CD8^+$ T-cells correlated negatively with infant $CD4^+$ T-cell percentage (Supplemental Figure 3, http://links.lww.com/QAD/D121).

There were no sex-based differences in CCR5+ $CD4^+$ or $CD8^+$ T-cell density or in the frequency of $CD4^+$ or $CD8^+$ T-cell subsets expressing immune activation (CCR5, ICOS and HLA-DR) or immune checkpoint molecules (TIGIT and PD-1) (data not shown).

Influence of maternal antiretroviral treatment

There were no significant differences in maternal CCR5 density, maternal or infant viral load, or sex of the infants born to transmitting mothers who did or did not receive ART during pregnancy, or in infant CCR5 density, maternal and infant viral load according to duration of ART (Supplemental Figures 4 and 5, http://links.lww.com/QAD/D121).

Comparison of CCR5 density on HLA-DR-, HLA-DR+, and TIGIT− and TIGIT+ subsets

We repeated the comparisons between the groups re-analysing CCR5 density on HLA-DR- and HLA-DR+ and on TIGIT− and TIGIT+ $CD4^+$ and $CD8^+$ T-cells, separately. CCR5 density was higher on HLA-DR+ than HLA-DR- $CD4^+$ and $CD8^+$ T-cells in both mothers and infants, as has been shown previously [35]. Additionally, CCR5 density was higher on TIGIT+ than on TIGIT- $CD4^+$ and $CD8^+$ T-cells in the infants (Fig. 2).

CCR5 density was higher in mothers who transmitted than in mothers who did not, and in infants with HIV-1 compared with infants without HIV-1 even on these subsets. Additionally, there was a larger spread of values for CCR5 density in mothers who transmitted and infants who acquired infection compared with mothers who did not transmit and infants without HIV-1 (Fig. 2).

After adjusting for maternal viral load and $CD4^+$ T-cell count, maternal CCR5 density on HLA-DR-, HLA-DR+, TIGIT− and TIGIT+ $CD4^+$ T-cells remained associated with transmission status. Similarly, infant CCR5 density on all infant subsets in the model, with the exception of CCR5 density on HLA-DR+ $CD8^+$ T-cells, remained associated with acquisition of infection after adjusting for maternal viral load and $CD4^+$ T-cell count (Supplemental Table 2, http://links.lww.com/QAD/D121).

Comparison of CCR5 density phenotypes in mother–infant pairs

Given that higher CCR5 density on both maternal and infant $CD4^+$ T-cells was associated with HIV-1 transmission/acquisition, we postulated that mother–infant pairs sharing a combined high CCR5 density phenotype would have the highest transmission risk compared with the opposite extreme of a shared low CCR5 density phenotype. To test this hypothesis, we divided mother–infant pairs into four groups according to levels of CCR5 density above (high) or below (low) the medians calculated for all mothers and all infants – high mother high infant (Hh), low mother high infant (Lh), high mother low infant (Hl) and low mother low infant (Ll). Mother–infant pairs in which the infant was infected were significantly more likely to have the Hh phenotype (51.9 vs. 8%) and the Lh phenotype (29.6 vs. 12%) and less likely to have the Ll phenotype (7.4 vs. 52%) ($P < 0.0001$) (Fig. 3). The highest odds ratio associated with HIV-1 infection in the infant was associated with the Hh phenotype (odds ratio = 45.5, 95% CI 5.6–372), but the Lh phenotype was also over represented (OR = 17.3, 95% CI 2.4–127) compared with Ll phenotype as the reference. Similar results were observed after adjusting for maternal viral load and $CD4^+$ T-cell counts (Table 2).

Discussion

Vertical transmission of HIV-1 is an ideal model for advancing our understanding of factors influencing both maternal transmissibility and infant susceptibility [36,37]. We hypothesized that CCR5 density on $CD4^+$ T-cells would be an important measure of risk of HIV-1 intrauterine transmission/acquisition. Compared with mothers who did not transmit HIV-1, those who transmitted HIV-1 had higher CCR5 density on $CD4^+$ and $CD8^+$ T-cells and higher frequencies of CCR5, ICOS, and TIGIT expressing $CD8^+$ T-cells. Similarly, compared with infants without HIV-1, infants with HIV-1 had higher CCR5 density on $CD4^+$ and $CD8^+$ T-cells, higher frequencies of CCR5, TIGIT, and PD-1 expressing $CD4^+$ and $CD8^+$ T-cells, and higher frequencies of HLA-DR+ $CD8^+$ T-cells. Furthermore, a combined maternal–infant high CCR5 density phenotype increased the risk of transmission/acquisition of in-utero HIV-1 infection.

Increased CCR5 density correlates with high viral loads in PWH [35] and high maternal viral load is the strongest risk factor for maternal–infant HIV-1 transmission [38]. The high maternal ART coverage in this study may have led to a larger proportion of early in-utero infections

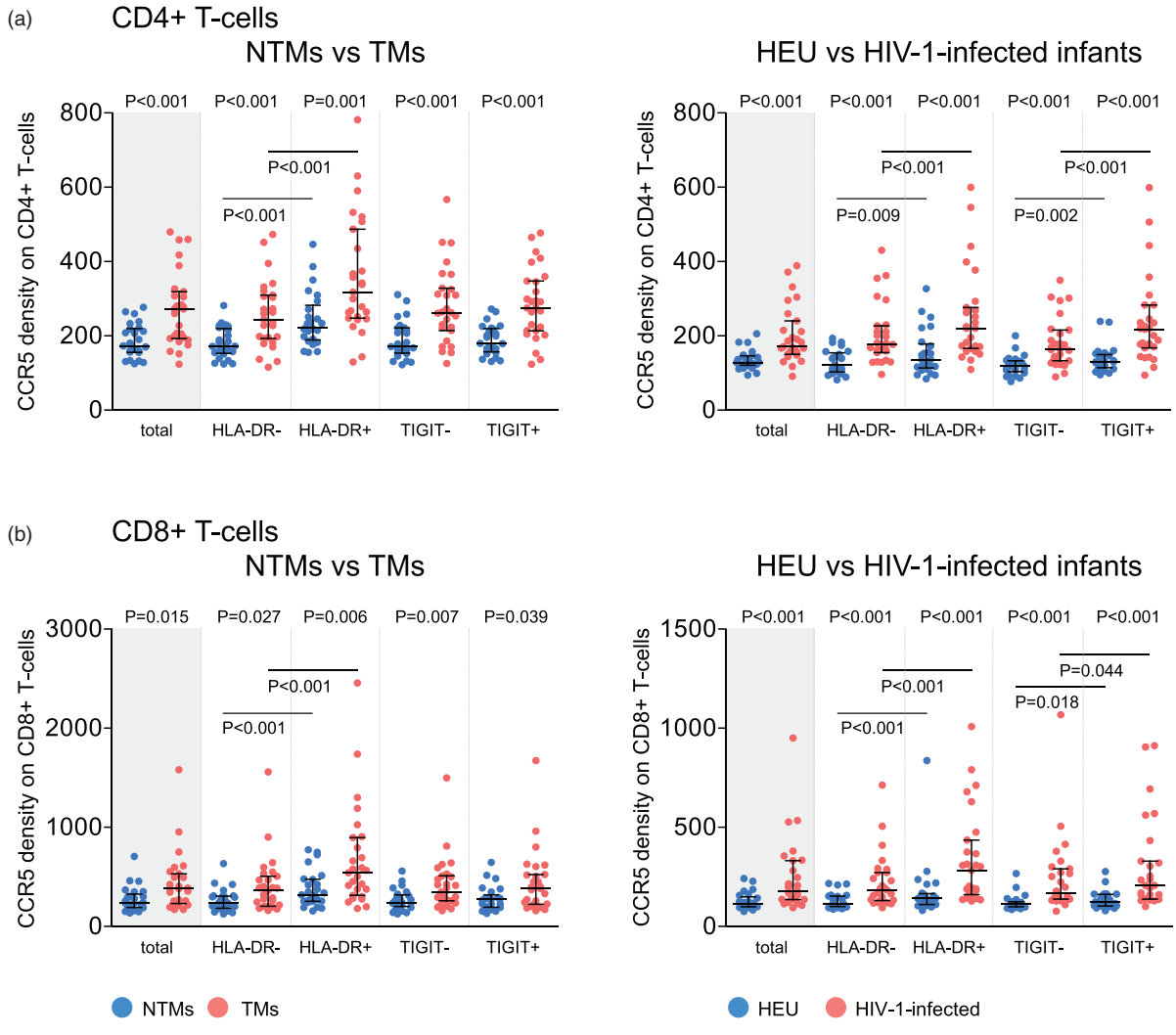

Fig. 2. CCR5 density on total CD4$^+$ and CD8$^+$ T-cells and HLA-DR−, HLA-DR+, TIGIT− and TIGIT+ CD4$^+$ and CD8$^+$ T-cells. CCR5 density was analysed on (a) total CD4$^+$ T-cells and HLA-DR−, HLA-DR+, TIGIT− and TIGIT+ CD4$^+$ T-cells in nontransmitting (NTMs) and transmitting mothers (TMs) (left panel) and HIV-exposed uninfected infants (HEU) and infants with HIV-1 (HIV) (right panel) and on (b) total CD8$^+$ T-cells and HLA-DR−, HLA-DR+. TIGIT− and TIGIT+ CD8$^+$ T-cells in NTMs and transmitting mothers (left panel) and HEU and HIV infants (right panel). Each symbol represents an individual. Horizontal lines and error bars represent the median, 25th and 75th percentiles. Significant *P* values shown compare CCR5 density from NTMs with transmitting mothers and HEU with HIV-1-infected infants as well as CCR5 density on HLA-DR− with HLA-DR+ and TIGIT− and TIGIT+ CD4$^+$ and CD8$^+$ T-cells. CCR5 density for total CD4$^+$ and CD8$^+$ T-cells is shaded in grey.

occurring before the initiation of ART during pregnancy. CCR5 density on CD4$^+$ T-cells remained associated with increased risk for in-utero HIV-1 transmission/acquisition even after adjusting for maternal viral load and CD4$^+$ T-cell count. The increased frequencies of ICOS+CD8$^+$ T-cells in the HIV-1 transmitting mothers suggests an association between maternal immune activation and increased risk of HIV-1 transmission as previously shown using CD38 as the activation marker [39]. In our study, ICOS was a stronger marker of activation in the mothers who transmitted than was HLA-DR.

As expected, infants with HIV-1 had higher levels of immune activation (HLA-DR+ CD8$^+$ T-cells) confirming previous studies [39–41]. This was not evident with ICOS as an activation marker. Our finding of higher levels of CCR5 in infants with HIV-1 corroborates studies showing that CCR5 density influences in-vitro infectibility [42] and that individuals with high CCR5 density on CD4$^+$ T-cells are more susceptible to HIV-1 infection [15].

When comparing the mothers to their infants, we observed that, as expected [43], CCR5 expression (density and frequency) was lower in infants compared with their mothers, regardless of infection status. CCR5 expression is constitutive, but immune activation related to environmental factors can increase this expression [44] and immune activation during pregnancy would likely

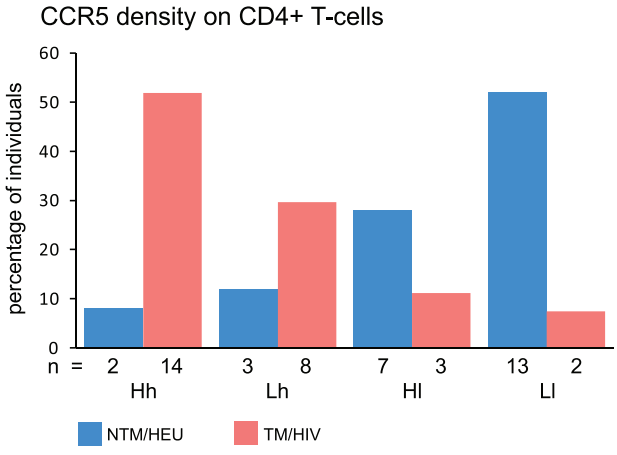

Fig. 3. Classification of NTM/HEU and TM/HIV mother–infant pairs into four CCR5 density groups. Based on CCR5 density on $CD4^+$ T-cells below (low) or above (high) the median for the mothers (NTMs and transmitting mothers combined) and the infants (HEU and HIV combined), the mother–infant pairs classified into four groups: high mother high infant (Hh), low mother high infant (Lh), high mother low infant (Hl) and low mother low infant (Ll). Sample numbers are shown.

impact CCR5 expression in both the mother and her infant. Interestingly, in the infants, CCR5 density on $CD4^+$ and $CD8^+$ T-cells, the frequency of CCR5+, TIGIT+, and PD-1+ CD4+ and $CD8^+$ T-cells, as well as the frequency of HLA-DR+$CD8^+$ T-cells, were associated with infection status. In the mothers, however, with the exception of CCR5 density on $CD4^+$ T-cells, the frequency of CCR5+, ICOS+, and TIGIT+ $CD8^+$ T-cells, but not $CD4^+$ T-cells, associated with transmission. The reason for this difference is unclear. $CD8^+$ T-cells may be more sensitive indicators of immune activation and immune perturbations than $CD4^+$ T-cells in mothers than in infants, possibly because the mothers have been infected for longer than the infants. Furthermore, in the infant, the extent of virus exposure and duration of infection in the presence of a developing immune system (a more tolerant environment) would be expected to influence the parameters measured.

Basal levels of CCR5 density are a determining factor in viral load [35]. As CCR5 expression can be modulated by cellular activation [17,45], Reynes *et al.* [17] defined basal levels of CCR5 density as levels on nonactivated HLA-DR- $CD4^+$ T-cells. Similarly to previous studies [17,44], we found that CCR5 density was higher on HLA-DR+ than HLA-DR- $CD4^+$ and $CD8^+$ T-cells for both groups of mothers and infants. When comparing CCR5 density on TIGIT+ to TIGIT− $CD4^+$ and $CD8^+$ T-cells, we found no difference between these subsets in the mothers. In the infants, however, CCR5 density was higher on TIGIT+ than TIGIT- $CD4^+$ and $CD8^+$ T-cells. Although CCR5 density measurements on total $CD4^+$ and $CD8^+$ T-cells includes both activated and nonactivated cells, CCR5 density remained elevated in the transmitting compared with the nontransmitting group when analysed on nonactivated cells only. Thus, regardless of HLA-DR expression, CCR5 density was higher in the mothers who transmitted compared with the mothers who did not, and in the infants with HIV-1 compared with those who remained uninfected. These findings suggest that activation is not solely driving the difference in CCR5 density and supports the hypothesis that CCR5 density is inherent and is genetically determined.

Interestingly, CCR5 density on $CD8^+$ T-cells was associated with reduced vertical transmission in model 2 (Table 1) in contrast with increased transmission observed in model 1. CCR5 density on $CD4^+$ and $CD8^+$ T-cells was highly correlated ($r = 0.848$, $P < 0.0001$). Thus, the association of CCR5 density on $CD8^+$ T-cells with vertical transmission (model 1) may be because of their correlation with CCR5 density on $CD4^+$ T-cells rather than a direct effect. The altered relationship of CCR5 density on $CD8^+$ T-cells with transmission (model 2) suggests that high CCR5 expression on $CD8^+$ T-cells, or likely a particular subset, might in fact be advantageous functionally and may be counteracting the effects of less functional exhausted $CD8^+$ T-cells in an environment marked by expanded frequencies of ICOS+ $CD8^+$ T-cells, which associated strongly with transmission.

As CCR5 density on $CD4^+$ T-cells significantly associated with vertical transmission, we rationalized that the combination of mother and infant CCR5 density phenotypes would further predetermine risk. The highest transmission risk was when both the mother and infant had the high CCR5 density phenotype (Hh) and

Table 2. Logistic regression results predicting transmitter status comparing low mother low infant (Ll) to high mother high infant (Hh), low mother high infant (Lh) and high mother low infant (Hl) unadjusted and adjusted for maternal viral load and $CD4^+$ T-cell counts.

	Unadjusted			Adjusted for maternal VL			Adjusted for maternal $CD4^+$ T-cell counts			Adjusted for maternal VL and $CD4^+$ T-cell counts		
Subset	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Ll	1.0	–	–	1.0	–	–	1.0	–	–	1.0	–	–
Hh	45.5	5.6–372	**<0.001**	28.8	3.2–263	**0.003**	60.3	6.3–580	**<0.001**	32.7	3.4–317	**0.003**
Lh	17.3	2.4–127	**0.005**	8.7	1–72	**0.045**	23.2	2.8–194	**0.004**	11	1.2–103	**0.036**
Hl	2.8	0.4–20.8	0.318	1.5	0.16–14	0.713	2.2	0.28–17	0.456	1.4	0.1–13	0.783

Statistically significant results are shown in bold. CI, confidence interval.

conversely the lowest risk was when both the mother and infant had the low CCR5 density phenotype (Ll). Interestingly, the risk of mother to infant transmission was higher when the mother had the low CCR5 density phenotype and the infant had the high-density phenotype (Lh) compared with when the mother had the high CCR5 density phenotype and the infant had the low-density phenotype (Hl) – suggesting that the infant's CCR5 density is more important than the mother's CCR5 density in vertical transmission and/or is modulated by HIV-1 infection.

Maternal ART during pregnancy dramatically reduces infant acquisition of HIV-1. The transmission rates in this population are less than 1% [46]. Because of this low transmission rate, the information obtained through studying our cohort of 27 in-utero infected infants is extremely valuable. The relatively small sample size likely lead to the wide confidence intervals observed when predicting transmitter status from the combined mother–infant pair CCR5 density phenotype. The CCR5 density results, however, are strong and convincing.

This study has a number of limitations. Information was not available regarding viral blips during pregnancy, time since infection or whether previous pregnancies resulted in infants with or without HIV-1.

It remains essential to improve our understanding of the mechanisms of perinatal HIV-1 transmission to enhance existing prevention interventions, develop new treatments, and contribute to finding curative approaches. Our identification of a role for a combined high CCR5 density phenotype in increasing the risk of transmission/acquisition of in-utero HIV-1 infection adds to the current body of knowledge of mechanisms involved in perinatal transmission and suggests that CCR5 inhibitors/antibodies, given during pregnancy, may be useful in decreasing vertical transmission. Our findings further support those host genetic studies, which highlight the importance of CCR5 expression levels in the acquisition of HIV-1 infection [8,10,47–49]. In addition, stem cell transplantation using CCR5 homozygote donors in PWH with various cancers also provides strong proof-of-principle of the importance of CCR5 as a target molecule in the context of HIV remission/cure [50–53]. How CCR5 expression during infancy might influence remission outcomes in early ART-treated paediatric cohorts deserves further exploration.

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Conflicts of interest

There are no conflicts of interest.

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