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Elevated Cytokine and Chemokine Levels in the Placenta Are Associated With *in utero* HIV-1 Mother-To-Child Transmission

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Abstract

Objective—To determine if there is an association between cytokine and chemokine levels in plasma isolated from the placenta and HIV-1 mother-to-child transmission (MTCT).

Design—We designed a case-control study of HIV-infected, pregnant women enrolled in the Malaria and HIV in Pregnancy cohort. Participants were recruited in Blantyre, Malawi from 2000-04. Cases were women whose children were HIV-1 DNA-positive at birth (*in utero* MTCT) or HIV-1 DNA-negative at birth and HIV-1 DNA-positive at 6-weeks post-partum (intrapartum MTCT); controls were women whose children were HIV-1 DNA-negative both at birth and 6-weeks post-partum.

Methods—After delivery, blood was isolated from an incision on the basal plate of the placenta. We used a Luminex assay to simultaneously quantify 27 cytokines, chemokines, and growth factors in placental plasma. HIV-1 RNA copies were quantified with the Roche Amplicor kit.

Results—Levels of IL-4, IL-5, IL-6, IL-7, IL-9, eotaxin, IL1Ra and IP-10 were significantly elevated in placental plasma isolated from cases of *in utero* HIV-1 MTCT. In contrast, only GCSF was elevated in placental plasma isolated from cases of intrapartum MTCT. After adjusting for maternal age, gestational age, and peripheral CD4+ T cell count, every log₁₀ increase in placental IP-10 was associated with a three-fold increase in the prevalence of *in utero* HIV-1 MTCT.

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Conclusions—Elevated cytokine and chemokine levels in placental plasma were associated with *in utero* and not intrapartum MTCT. IP-10, which is both a T-cell chemokine and potentiator of HIV-replication, was robustly and independently associated with prevalent, *in utero* MTCT.

Keywords

Human immunodeficiency virus type 1 (HIV-1); mother-to-child transmission (MTCT); placenta; sub-Saharan Africa; chemokines; IP-10

INTRODUCTION

In populations where replacement feeding is unfeasible, it has been estimated that between 25-35% of children born to HIV-infected pregnant women will become HIV-infected [1]. Of these mother-to-child transmission (MTCT) events, estimates suggest that in a breast-fed population, 25 to 35% of MTCT occurs *in utero* prior to delivery, 40 to 55% occurs during labor and delivery, and the remaining 25 to 35% occurs during breastfeeding [1]. Numerous studies have established that high maternal HIV-1 load is a major risk factor for HIV-1 MTCT [2-6], and reduction of maternal HIV-1 RNA load with antiretroviral drugs significantly reduces the rate of HIV-1 MTCT [7, 8]. Additional factors such as chorioamnionitis [9], syphilis [6] and possibly placental inflammation [9-11], have also been associated with HIV-1 MTCT. Although the timing of HIV-transmission is fairly clearly understood, apart from the aforementioned conditions, the molecular mechanism of HIV-1 MTCT is not well understood.

Previous studies from our group indicated that the HIV-1 RNA concentration in placental blood differs by HIV-1 vertical transmission status and that HIV-1 replicates locally in the placental environment [12]; in the same study, we also observed phylogenetic evidence of HIV-1 compartmentalization in placentas isolated from women who transmitted HIV-1 *in utero*. Viral compartmentalization in the placenta suggests that HIV-1 can adapt to the placental microenvironment. One of the key components that may regulate HIV-1 replication in the placenta is the localized immune state. The maternal-placental interface is a unique immune environment characterized by a strong infiltrate of cells including natural killer (NK) cells, macrophages, and dendritic cells [13]. These immune cells as well as resident placental cells such as trophoblasts are known to secrete a variety of cytokines, chemokines and growth factors [14]. The influences of these factors on both HIV-1 replication *in vivo* and HIV-1 MTCT are not fully understood.

Studies on sexual transmission indicate that innate immune defenses play a crucial role in transmission of SIV and HIV-1 by compromising the integrity of the vaginal mucosal barrier due to associated inflammation and by increasing target cell availability [15]. A microenvironment facilitating transmission may be created by chemokines (MIP class) and pro-inflammatory cytokines (GM-CSF, IL-1, IL-6 and IL-8) that recruit neutrophils, dendritic cells, macrophages and lymphocytes [16, 17]. Cytokines also play an important role in the early stages of viral infection by exhibiting direct antiviral activity, activating and recruiting various immune effector cells, and modulating adaptive antiviral immune responses [18, 19]. Some cytokines and chemokines can up-regulate HIV-1 expression in target cells, regulate the availability and activity of transcription factors that bind to the promoter of HIV-1 provirus, enhance expression of target cell receptors and recruit and activate resting CD4+ T lymphocytes [20-25].

Though several groups have addressed the effect of specific cytokine responses in acute HIV-1 infection during sexual transmission [21, 26, 27], only limited studies have addressed how placental innate immunity may modulate HIV-1 MTCT [28, 29]. We designed an

exploratory study to interrogate cytokine, chemokine and growth factor levels in placental plasma isolated from 84 HIV-1 non-transmitting (NT), 35 *in utero* (IU) and 34 intrapartum (IP) transmitting mothers. The overall objective of this study is to determine if immune modulation in the placental milieu is associated with HIV-1 mother to child transmission.

MATERIAL & METHODS

Study Participants and Clinical Isolates

All participants gave written, informed consent to participate. Ethical approval for the parent Malaria and HIV in Pregnancy (MHP) observational cohort was obtained from the University of North Carolina at Chapel Hill Institutional Review Board (IRB) and the Malawi College of Medicine Research Ethics Board (COMREC); approval for the present case-control study, which used only de-identified information, was obtained from The Ohio State University. Blood samples were collected as part of the MHP cohort, which enrolled 3,824 pregnant women admitted to the Labor and Delivery ward of Queen Elizabeth Central Hospital in Blantyre, Malawi from 2001-04 [6, 30]. Women were ineligible for MHP if they were in the active phase of labor, were participating in other research studies, were less than 15 years of age, had hypertension, multiple gestations or altered consciousness. Maternal HIV-1 status was determined by two rapid tests, as described previously [30]. Pregnant women with acute HIV-infection [31] were excluded from this study. Women who delivered stillbirths or died at delivery were also excluded from the study. At the onset of active labor, women received a single dose of nevirapine and shortly after birth, infants received a single dose of nevirapine, both according to the HIVNET 012 protocol [8]. Infant HIV-1 status was determined by real-time polymerase chain reaction (PCR) testing according to the methods of Luo *et al.* [32], and pediatric HIV-infection was categorized according to standard definitions [33], as follows: HIV-1 transmission cases were classified as IU transmissions if infants were HIV-1 DNA positive within 48 hours of birth; classified as IP transmissions if infants were HIV-1 DNA negative at birth and HIV-1 DNA positive 6 weeks post-partum; and as HIV-negative, or non-transmitters (NT) if infant samples from both birth and six weeks post-partum were HIV DNA negative. Overall, 1157 (30%) of the pregnant women in MHP were HIV-1 infected, with 73 (9%) of their newborns HIV-1 infected IU and 66 (13%) of their infants infected IP [6]. Maternal hemoglobin concentration was determined by Hemocue hemoglobinometer (HemoCue AB, Ängelholm, Sweden); CD4⁺ T-cells were quantified by FACScan (Becton Dickinson, California); HIV-1 RNA was quantified from peripheral and placental plasma using Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics, Branchburg, NJ), with plasma HIV-1 RNA concentrations <400 assigned a value of 400 copies/mL. Peripheral malaria was diagnosed using Geimsa-stained thick and thin blood smears and placental malaria was diagnosed based on placental histology, both as described in Mwapasa *et al.* [30]. Syphilis was diagnosed using the Rapid Plasma Reagin test ([RPR], Omega Diagnostics, Alloo, Scotland), and all RPR reactive sera were tested with the *Treponema pallidum* Hemagglutination Assay ([TPHA], Omega Diagnostics, Alloo, Scotland). Participants with a reactive RPR followed by a reactive TPHA were considered syphilis seroreactive, as described [34].

Study design

Placental plasma was available from 35 women whose infants became HIV-infected *in utero* and 34 women whose infants became infected intrapartum. Owing to the exploratory nature of the study, the number of NT samples in the control group was selected based on the number of assays available per 96-well plate (n=84). The 84 NT samples were randomly chosen, using STATA (version 10.1, StataCorp, College Station, Texas, United States), from the total collection of NT samples with placental plasma available. By design, NT samples were not matched with either IU or IP samples. The participants included in this study were

similar to those not included with regard to maternal age, CD4+ T-cell count, and peripheral HIV concentration (Wilcoxon rank-sum test, all p-values greater than 0.3). When stratified by HIV-transmission status (NT, IU, IP), included and excluded participants were also similar, with the exception of peripheral viral load in the IU subgroup: the included IU samples had a lower peripheral viral load than those not included (4.7 log₁₀ copies/mL versus 5.1 log₁₀ copies/mL, Wilcoxon rank sum test p=0.08).

Collection of placental plasma

After delivery of the placenta, an incision was made in the middle of the maternal surface of the placenta, two cm long and through half the thickness of the placental tissue. The placental blood that pooled in the incision was collected, and plasma was prepared according to standard protocols.

Luminex cytokine assay

Twenty-seven analytes were simultaneously quantified from 20 µL of placental plasma with a Bio-Plex Pro Human Cytokine 27-plex kit (BioRad, Hercules, CA) according to the manufacturers instructions. The analytes include the following: IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1(MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF. Data were acquired using a Bio-Plex-200 suspension array system and the cytokine/chemokine concentrations were automatically calculated with Bio-Plex Manager software 5.0 using a standard curve derived from a recombinant cytokine standard (supplied by the manufacturer). When a limited number of the data were outside of the range of the standard curve, the values were set to the maximum or minimum value of the standard curve. The majority of the FGF basic, MIP-1α, VEGF, and RANTES data were outside of the range of the standard curve, and therefore they were excluded from further analysis.

Evaluation of placental biopsies

A subset of the participants had representative formalin-fixed, paraffin-embedded full-thickness placental biopsies available. Routine tissue sections were cut from placental biopsies and stained with Hematoxylin and Eosin (H&E), according to standard protocols. Histopathological analysis of placental sections was performed by an expert placental pathologist (NCR). Light microscopy evaluation was performed using standard objectives (4X, 10X, 20X and 40X). The presence or absence of histological placental parenchymal abnormalities was noted, including: edema, increased syncytial knots, changes in the villous vasculature, placental maturity, and non-specific acute and/or chronic villitis and/or intervillitis. Histological evidence of infection with the ToRCHES microbes (Toxoplasma, Rubella, Cytomegalovirus, Herpes, Enteroviruses, and Syphilis), which are known to cross the maternal-fetal barrier, was also assessed. Chorioamnionitis was assessed by a single pathologist (DAM) according to methods previously described [35]. Briefly, H&E stained sections of cord, membranes, and placenta and/or Giemsa stained sections of placenta were evaluated under light microscopy for the presence of neutrophils in the chorion, amnion, umbilical cord, and fetal plate. Both pathologists were masked to the HIV-1 transmission status of the participants.

Statistical analysis

Statistical analysis was performed with STATA and SAS (Version 9.2, Cary NC). The Wilcoxon rank-sum test or the Fisher's exact test was used to compare clinical and virological features between the transmission groups. Univariate associations were tested with a two-sample Wilcoxon rank-sum test, with IU and IP considered as separate outcomes. As this is an exploratory study, we did not correct for multiple hypothesis testing [36] except

as a sensitivity analysis of our main findings. In that case we applied a Bonferroni correction (p-value cutoff = 0.05 divided by 23 analytes equals an adjusted p-value cutoff of 0.002). To further explore the association between IP-10 and IU MTCT, we used regression models specifying the Poisson distribution, with IP-10 as the primary exposure and transmission status (IU versus NT) as the outcome; IP transmission events were excluded from the multivariable modeling analyses. All cytokine measures were $\log_{10}$ transformed prior to modeling. The exposure variable and all covariates met the assumption of linearity in the log risk. Based on previous literature and preliminary analysis, we assessed maternal age, gestational age, CD4+ T-cell count, hemoglobin level, maternal temperature at delivery, IL-4, IL-5, IL-6, IL-7, IL-9, eotaxin, and IL-1Ra as potential confounders. The full model was reduced using manual backward elimination [37]. We assessed potential confounders one by one and retained those variables that changed the main effect estimate by more than 10% when removed from the full model.

RESULTS

Participant characteristics

We designed a case-control study to compare the concentration, in placental blood, of several chemokines, cytokines, and growth factors stratified by HIV-1 mother-to-child transmission status. In total, 153 placental plasma isolates were included in the analysis: 35 from cases of *in utero* transmission (IU MTCT), 34 from cases of intrapartum transmission (IP MTCT), and 84 from cases of HIV-exposed but uninfected (non-transmitters, NT). The three groups were balanced for most maternal characteristics, with the exception of gestational age (slightly lower among cases of IU MTCT) and both peripheral and placental viral load (higher among both IU and IP MTCT cases) (Table 1).

Multiple cytokines and chemokines are associated with *in utero* HIV-1 MTCT

The primary objective of this study was to compare analyte levels in placental blood isolated from mother-infant pairs with known HIV-transmission status (Table 2). Median analyte levels in each of the transmission categories were consistent with previously published range of analyte concentrations [27]. Owing to the likelihood that IU and IP MTCT occur via different biological mechanisms, we analyzed the data as two separate case-control studies, NT versus IU and NT versus IP. Eight analytes in placental blood were significantly higher in cases of *in utero* transmission compared to non-transmitters (Table 2 and Figure): IL-4 (36%), IL-5 (200%), IL-6 (56%), IL-7 (35%), IL-9 (218%), eotaxin (162%), IL-1Ra (190%), and IP-10 (252%). In contrast, only GCSF was elevated during IP MTCT, by 159%. After correcting for multiple comparisons, only the association between IP-10 and IU MTCT remained statistically significant. These data suggest that the placental environment, specifically the cytokines and chemokines present in the placenta, differs by both HIV-1 transmission status and by mode of transmission.

We examined a matrix of correlation coefficients to evaluate the association between CD4+ T cell count, HIV-1 RNA concentration and the chemokines and cytokines that were associated with IU MTCT (Table 3). Although several of the analytes were strongly correlated with each other, none was strongly or significantly associated with CD4+ T cell count. IP-10 was significantly yet weakly correlated with peripheral HIV-1 RNA level and strongly correlated with placental HIV-1 RNA level. Participants with placental malaria had higher levels of IP-10 in placental plasma than participants without evidence of placental malaria (2.4 versus 2.7 $\log_{10}$ pg/mL IP-10, $p=0.05$, Wilcoxon rank-sum test). Previous studies have documented an association between IP-10 levels in amniotic fluid and chorioamnionitis, but we observed no correlation between IP-10 concentration in placental plasma and chorioamnionitis ($p=0.3$, two-sample Wilcoxon rank-sum test).

Placental histology

In order to exclude microbial infection of the placenta, we analyzed placental biopsies for evidence of histological abnormalities. A total of 94 biopsies was available for analysis: 56/84 NT (67%), 17/35 IU (49%), and 21/34 IP (62%). This subset of placental biopsies was a convenience sample and it did not differ from the parent population in regard to peripheral HIV-1 concentration, CD4+ T-cell count, gestational age, maternal age, IL-4, IP-10, or IFN- γ levels (data not shown). We observed no significant microscopic placental pathological findings associated with IU MTCT, including those traditionally associated with malaria or ToRCHES infections. However, edema, either in the placenta or in the fetal membranes, was observed in 10 NT (20%), 6 IU (38%), and 10 IP (50%) biopsies (NT versus IU, $p=0.2$; NT versus IP, $p=0.02$; Fisher's exact test). We identified several curious findings, including a less-than-typical amount of fibrin in the intervillous space and a high prevalence of syncytial knots, although neither feature was associated with HIV-1 transmission (data not shown).

Regression analysis

Our preliminary analyses suggested that IP-10 may be meaningfully associated with IU MTCT. In a univariate Poisson regression analysis, every $\log_{10}$ increase in IP-10 increased the prevalence of IU MTCT by 70% (prevalence ratio (PR): 1.7, 95% confidence interval [CI]: 1.3, 2.3). We built a multivariate Poisson regression model with IU MTCT as the dependent variable, IP-10 as the primary exposure variable of interest, and all covariates found to be significant in univariate analyses as potentially confounding variables. After backward elimination, gestational age, IL-4, IL-5, IL-7, and IL-1Ra were retained as confounders (Table 4). Maternal age and CD4+ T-cell count were kept in the final regression model for *a priori* considerations even though they did not meet the 10% criterion for confounding. The remaining variables (temperature, hemoglobin, IL-6, IL-9, and eotaxin) were not retained. After adjustment, every one- $\log_{10}$ increase in IP-10 increased the prevalence of IU MTCT more than 3-fold (PR: 3.3, (95% CI: 1.3, 8.1). We conducted two sensitivity analyses: a) including placental viral load in place of CD4+ T-cell count in the final model; and b) including peripheral viral load in place of CD4+ T-cell count in the final model. (Models containing CD4+ T-cell count together with either viral load variable did not converge due to collinearity). Both viral load variables had considerable missing data, leading to a loss of 37% and 12% of subjects, respectively, when placental or peripheral viral load were used in place of CD4+ T-cell count. For the model containing placental viral load, the PR for IP-10 was similar to the primary analysis (PR: 3.6, 95% CI: 1.0, 13). For the model containing peripheral viral load, the PR for IU MTCT was again similar at 3.7 (95% CI: 1.2, 11). Both sensitivity analyses indicated that use of CD4+ T-cell count instead of viral load did not meaningfully change the magnitude of the observed effect of IP-10 on IU MTCT, though precision of the estimate after adjustment for viral load suffered because of missing data leading to smaller sample size.

DISCUSSION

The objective of this exploratory case-control study was to determine if the levels of chemokines, cytokines, or growth factors in placental plasma were associated with HIV-1 mother-to-child transmission. We observed the following: (1) IL-4, IL-5, IL-6, IL-7, IL-9, eotaxin, IL-1Ra, and IP-10 levels and HIV-1 RNA concentration were higher in placental plasma from IU MTCT than in placental plasma from NT controls (2) only GCSF was elevated in placental plasma prepared from IP MTCT cases compared to placental plasma from NT controls, and (3) after adjustment for confounding variables and either CD4+ T-cell number or viral load, multivariate modeling indicated that IP-10 levels were independently associated with a three-fold increase in the prevalence of IU MTCT. These

observations suggest that an altered immune environment in the placenta is associated with IU MTCT and not IP MTCT.

Many of the analytes elevated in placental plasma during IU MTCT have previously been associated with HIV-1 infection. For example, IL-1Ra, an inducible receptor antagonist of IL-1, is known to be elevated in inflammatory diseases, including HIV-1, where it may serve as a marker of disease progression [38-41]. Increased levels of IL-7 is consistent with other studies, however these studies also found increased IL-7 in correlation with the level of CD4+ T cell depletion suggesting the role of IL-7 in T-cell apoptosis and hence disease progression [42-44]. However, a similar association between IL-7 and CD4+ T cell count was not observed in these samples (Table 3). IL-6 is considered a pro-inflammatory mediator and it has been shown to induce HIV-1 replication by both transcriptional and post-transcriptional mechanisms [45]. On the other hand, HIV-1 infection is also known to induce IL-6 [46, 47], which confounds our ability to interpret the association between IU MTCT, IL-6, and HIV-1 RNA concentration in placental blood. IL-4 is an important regulator and marker of accelerated HIV-1 disease progression, and it has been shown to collaborate with HIV-1 to induce IL-6 production, thereby resulting in global B cell dysfunction [48, 49]. Finally, IL-5 and eotaxin can both recruit eosinophils, which have been shown to be permissive targets for HIV-1 replication [50, 51].

The observed changes in multiple placental cytokine/chemokine profiles during IU MTCT is in stark contrast to the placental cytokine/chemokine profile during IP MTCT, in which only GCSF level was elevated. The observed contrast in cytokine levels between *in utero* and intrapartum MTCT provides additional data to support the hypothesis that IU and IP MTCT have different molecular mechanisms. Consistent with this hypothesis, we (and others) have previously observed no association between maternal-fetal blood admixture (also called placental microtransfusions) and IU MTCT [52-54]; in contrast, using two different assays, we have observed an association between placental microtransfusions and IP MTCT [52, 53]. Whether or not the prevalent edema that was associated with intrapartum MTCT is related to placental microtransfusions cannot be determined from this study.

IP-10, also called CXCL10, is a type 1 chemokine that binds to the CXCR3 receptor [55] and attracts Th1 lymphocytes, monocytes, eosinophils, and dendritic cells, many of which are permissive to HIV-1 infection and replication. In this study, IP-10 was significantly correlated with peripheral and placental HIV-1 RNA concentration, and IP-10 levels were independently correlated with IU MTCT. IP-10 has been shown to increase HIV-1_{BAL} replication in monocyte-derived macrophages and peripheral blood lymphocytes [56], which could explain the elevated HIV-1 RNA concentration in placentas obtained from IU MTCT cases; to our knowledge, data demonstrating a direct affect of IP-10 on HIV-1 replication in the placental microenvironment has not been reported. Given the sequence variability within the HIV-1 promoter [57], further studies are needed to determine if all HIV-1 variants replicate similarly when IP-10 signaling cascades are activated. Other studies have shown that IP-10 levels in peripheral blood are transiently induced during the acute stage of HIV-infection [27] and during untreated, progressive HIV -infection [58]. Malaria infection has been associated with both increased levels of IP-10 [59, 60] and leukocyte infiltration into the placenta [61, 62], each of which could potentially create an environment favoring HIV-replication. In this study, we observed a small yet significant association between placental malaria and placental IP-10 levels; as we have previously reported in the parent MHP cohort, placental malaria was associated with an increased HIV-1 concentration in the placenta [30] but it was not associated with an increased prevalence of HIV-1 MTCT [6].

Multiple cell types secrete IP-10, including keratinocytes, endothelial cells, macrophages, and fibroblasts in the adult [63] and Hofbauer cells, stromal cells, and endothelial cells on

the fetal side of the maternal-fetal interface [64]. Preterm infants can have transiently elevated IP-10 levels [65]. Although we interpret cytokine and chemokine levels in placental blood to reflect their concentration in maternal blood in the intervillous space, it is also possible that the method of blood collection used in this study (incision) created maternal-fetal blood admixture [66]. Only by repeating the cytokine/chemokine arrays on umbilical cord blood could we conclusively determine if the data reflects the cytokine storm in the fetus. Unfortunately, this type of specimen is not available from this study. Several observations from our previous study [12], which used samples from the same cohort obtained using the same method of placental blood collection, argue against placental blood representing only fetal blood. First, we sequenced HIV-1 envelope genes from peripheral blood, placental blood, and umbilical cord blood, and the collection of *env* genes in both maternal and placental blood were highly diverse, in stark contrast to the nearly homogenous viral population in cord blood. If the placental blood only represented fetal blood, we would expect it to have minimal viral sequence diversity. Second, phylogenetic reconstruction identified a fetal compartment of HIV-1 that was distinct from the placental compartment [12]. However, even if our findings reflect the cytokine/chemokine profile following acute fetal HIV-infection, they are consistent with a previous report of elevated plasma IP-10 in plasma donors acutely infected with HIV-1 [27].

In summary, this study supports the association between elevated levels of specific cytokines and chemokines in placental plasma and IU, but not IP, MTCT. IP-10 levels were independently associated with IU MTCT, and this association remained after controlling for maternal immune status and other confounding variables. These data may provide a direct link between the placental immunological state and viral replication, either through direct enhancement of HIV transcription or via localized recruitment of cells that are permissive to HIV-replication.

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Figure. Elevated levels of cytokines and chemokines are associated with *in utero* HIV-1 mother-to-child transmission

Analyte concentration was measured in placental plasma isolated from 84 HIV-infected non-transmitters (NT), 35 cases of *in utero* (IU) mother-to-child transmission (MTCT), and 34 cases of intrapartum (IP) MTCT. For each of the indicated analytes, IU and IP data were independently compared to NT data using a Wilcoxon rank-sum test. Dots represent individual measurements and the horizontal line represents the median value.

TABLE 1

Participant Characteristics

Characteristic	Nontransmitter (N=84)	In utero transmitter (N=35)	Intrapartum transmitter (N=34)	p-value [*] NT vs. IU	NT vs. IP
Age, years (median, IQR)	25 (21, 29)	24 (20, 26)	24 (21, 30)	0.4	0.9
CD4+ T cell count [†] , cells/ μ L (median, IQR)	373 (223, 495)	284 (113, 408)	337 (163, 511)	0.08	0.6
Chorioamnionitis [‡]	29%	38%	41%	0.4 ^{**}	0.3 ^{**}
Duration of labor [‡] (hours) (median, IQR)	9 (3, 14)	11 (3, 14)	8 (5, 16)	0.9	0.9
Duration of membrane rupture [‡] , minutes (median, IQR)	85 (20, 244)	100 (10, 430)	105 (25, 255)	1.0	0.8
Gestational age (estimated), weeks (median, IQR)	40 (38, 40)	38 (37, 39)	39 (37, 40)	0.001	0.01
Malaria (peripheral) [‡]	10%	11%	9%	0.7 ^{**}	1.0 ^{**}
Malaria (placental) [‡]	11%	18%	19%	0.4 ^{**}	0.2 ^{**}
Syphilis seroreactive	6%	17%	6%	0.08 ^{**}	1.0 ^{**}
Temperature, °C (median, IQR)	36 (36, 37)	36 (36, 37)	37 (36, 37)	0.7	0.7
HIV-1 [RNA] (periphery) [‡] , log ₁₀ copies/mL (median, IQR)	4.3 (3.8, 4.9)	4.7 (4.1, 5.1)	4.9 (4.4, 5.3)	0.04	0.005
HIV-1 [RNA] (placenta) [‡] , log ₁₀ copies/mL (median, IQR)	3.7 (2.9, 4.1)	5.1 (4.1, 5.9)	4.4 (4.0, 4.9)	<0.0001	0.007

^{*} Unless noted otherwise, p-values are from the Wilcoxon rank-sum test

^{**} P-value from Fisher's exact test

[†] Missing data. CD4 T-cell count data were available from 82 NT, 34 IU MTCT and 34 IP MTCT; Chorioamnionitis data were available from 72 NT, 34 IU MTCT and 32 IP MTCT; Duration of labor data were available from 77 NT, 30 IU, and 29 IP; Duration of membrane rupture data were available from 71 NT, 29 IU, and 27 IP MTCT; Malaria (placental) data were available from 84 NT, 34 IU MTCT and 31 IP MTCT; HIV RNA (periphery) data were available from 71 NT, 26 IU MTCT, and 27 IP MTCT; HIV RNA (placenta) data were available from 40 NT, 15 IU MTCT, and 12 IP MTCT.

TABLE 2

Analyte levels in the placenta differ by HIV transmission status

Analyte	Concentration, ** median (IQR) *, (pg/mL)			p-value [†]	
	NT*	IU*	IP*	NT versus IU MTCT*	NT versus IP MTCT
IL-1β	0.38 (0.11, 1.2)	0.58 (0.11, 2.2)	0.88 (0.12, 0.88)	0.4	1.0
IL-2	0.82 (0.25, 2.8)	1.51 (0.25, 5.5)	0.25 (0.06, 2.3)	0.8	0.1
IL-4	0.41 (0.23, 0.70)	0.56 (0.35, 0.98)	0.83 (0.21, 0.83)	0.04	0.4
IL-5	0.16 (0.04, 0.41)	0.32 (0.13, 0.79)	0.40 (0.04, 0.40)	0.03	0.7
IL-6	9.6 (3.4, 32)	15 (7.4, 33)	16 (6.3, 58)	0.04	0.1
IL-7	0.74 (0.44, 1.4)	1.0 (0.69, 2.0)	0.66 (0.34, 1.3)	0.02	0.4
IL-8	1.9 (0.04, 13)	4.9 (0.34, 18)	4.1 (0.34, 26)	0.2	0.2
IL-9	9.6 (3.4, 18)	21 (3.6, 32)	9.0 (4.0, 15)	0.04	0.6
IL-10	0.55 (0.07, 1.5)	1.2 (0.07, 2.8)	0.21 (0.07, 2.0)	0.2	0.7
IL-12(p70)	3.9 (1.8, 6.4)	5.5 (1.2, 10)	3.7 (2.4, 7.9)	0.2	0.6
IL-13	0.58 (0.23, 0.95)	0.83 (0.16, 1.2)	0.67 (0.19, 0.9)	0.3	0.8
IL-15	0.33 (0.05, 2.0)	0.26 (0.05, 1.7)	0.17 (0.05, 1.3)	0.9	0.2
IL-17	6.4 (1.6, 13)	4.4 (1.1, 19)	3.9 (0.31, 12)	0.8	0.2
Eotaxin	6.8 (4.4, 11)	11 (4.7, 17)	7.7 (4.6, 11)	0.02	0.7
GCSF	6.9 (4.8, 10)	11 (5.1, 21)	11 (7.0, 20)	0.1	0.04
GMCSF	5.9 (2.5, 11)	6.5 (2.3, 20)	4.4 (0.59, 9.8)	0.1	0.1
IFN-γ	18 (9.7, 38)	35 (11, 71)	12 (5.2, 26)	0.1	0.08
MCPI-MCAF	3.5 (1.2, 8.3)	4.7 (1.4, 16)	2.7 (0.68, 12)	0.6	0.6
MIP1	41 (23, 68)	50 (30, 67)	46 (24, 65)	0.3	0.7
TNF-α	24 (12, 50)	22 (6, 61)	25 (11, 38)	0.9	0.7
IL1Ra	58 (33, 108)	110 (48, 210)	73 (40, 120)	0.03	0.5
IP-10	210 (140, 430)	530 (270, 1100)	350 (140, 1000)	0.002	0.2
PDGFB	1800 (490, 3900)	2100 (610, 4600)	1400 (730, 4900)	0.4	0.7

* IQR= interquartile range, NT= nontransmitter, IU= *in utero* transmission, IP=intrapartum transmission, and MTCT= mother-to-child transmission.

** Analyte concentrations rounded to two significant figures, p-values rounded to one significant figure.

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[‡] Analyte values in the transmission groups were compared using a Wilcoxon rank-sum test.

TABLE 3

Correlation of analytes, CD4+T-cell count, and HIV RNA concentration.

	CD4+ T-cells [†]	HIV (peri) [*]	HIV (plac) [*]	IL-4	IL-5	IL-6	IL-7	IL-9	Eotaxin	IP-10
HIV-1 (peri.)	-0.40	*								
HIV-1 (plac.)	-0.32	0.39	*							
IL-4	0.10	-0.05	0.09	*						
IL-5	0.01	-0.02	-0.05	0.64	*					
IL-6	-0.17	0.18	0.54	0.08	-0.08	*				
IL-7	0.12	-0.05	0.14	0.79	0.65	0.17	*			
IL-9	0.07	0.02	0.16	0.68	0.34	0.21	0.64	*		
Eotaxin	0.14	0.004	0.13	0.53	0.21	0.33	0.57	0.72	*	
IP-10	-0.07	0.24	0.54	-0.02	-0.18	0.45	0.13	0.27	0.45	*
IL-IRa	-0.02	0.12	0.35	0.43	0.10	0.55	0.47	0.56	0.68	0.54

* HIV (peri) = HIV-1 RNA copies/mL in peripheral blood; HIV (plac) = HIV-1 RNA copies/mL in placental blood.

** Values represent Spearman's rank correlation coefficient. All values, except CD4⁺T-cells are log₁₀ transformed. Grey shading indicates p<0.05.[†] Included data. CD4+ T cells n=148, HIV(peri) n=122, HIV(plac) n=64, and all other correlations n=151.

TABLE 4

Multivariable assessment of placental IP-10 and prevalence of IU MTCT.

	PR	(95% CI)
IP-10 **	3.3	(1.3, 8.1)
Gestational age (weeks)	0.77	(0.64, 0.93)
CD4+Tcells (cells/ μ L)	1.0	(1.0, 1.0)
maternal age (years)	0.98	(0.90 1.1)
IL-4 **	5.0	(0.73, 34)
IL-5 **	1.7	(0.79, 3.7)
IL-7 **	0.41	(0.08, 2.0)
IL-1Ra **	0.58	(0.24, 1.4)

* PR = prevalence ratio; CI = confidence interval

** Log₁₀ transformed prior to multivariable analysis.