HIV-related immune activation attenuates polyfunctional IgG and memory B-cell responses to Tdap immunization during pregnancy

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Summary

Background Maternal pertussis vaccination with Tdap vaccine is recommended to protect newborns from severe postnatal infection. HIV-exposed uninfected (HEU) infants have a higher incidence of pertussis infection and may particularly benefit from maternal immunization. The impact of HIV infection on the quality of IgG and memory B cell (MBC) responses to Tdap vaccination in pregnant women (PW) living with HIV (PWH) is unknown.

Methods In this observational study, humoral immune responses to Tdap vaccination, including IgG levels, Fc-dependent effector functions, and MBC frequencies, were measured before and after vaccination in 40 PWH and 42 HIV-uninfected PW. Placental transfer of IgG and avidity were assessed in cord blood (CB). Soluble and cellular immune activation markers were quantified at baseline.

Findings One month after vaccination, PWH had lower frequencies of MBC compared with HIV-uninfected PW. At delivery, PWH had attenuated pertussis-specific IgG levels and Fc-dependent effector functions. Reduced levels of maternal vaccine polyfunctional IgG and IgG avidity were transferred to HEU as compared to HIV-unexposed newborns. After adjustment with ethnicity, maternal antibody levels and gestational age at vaccination, HIV infection was independently associated with decreased levels of PT specific-IgG in CB. Both maternal and neonatal pertussis-specific IgG responses as well as PT-specific IgG avidity were inversely correlated with maternal sCD14 levels before vaccination among PWH.

Interpretation Maternal HIV infection is associated with attenuated humoral immune responses to Tdap vaccination that correlate with sCD14. Suboptimal transfer of maternal immunity may further increase the risk of severe pertussis infection in HEU infants.

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Research in context

Evidence before this study

HIV-exposed uninfected (HEU) newborns have a higher incidence of pertussis infection than HIV-unexposed infants. Pertussis immunization during pregnancy with tetanus diphtheria acellular pertussis (Tdap) vaccine is an effective strategy recommended to protect newborns from severe postnatal infection. Chronic immune activation and altered B-cell profiles are observed in HIV infection. Evidence suggests that maternal HIV infection is associated with lower levels of protective vaccine-specific antibodies and reduced efficiency of transplacental antibody transfer for immunization against influenza. The impact of maternal HIV infection on humoral responses to pertussis immunization has not yet been characterized.

Added value of this study

Our data indicate that pregnant women living with HIV (PWH) with a long history of antiretroviral therapy (ART) uptake and undetectable viral load show evidence of attenuated humoral immune response to Tdap immunization. This results in distinct pertussis-IgG immunity in HEU newborns, as evidenced by the lower level and quality of IgG. This reduction in antibody-dependent immunity correlated with sCD14 and an abnormal CD4/CD8 ratio at the time of vaccination.

Implications of all the available evidence

These findings highlight that attenuated immune response in PWH and suboptimal transfer of vaccine-induced maternal immunity may contribute to decreased protection against pertussis infection in HEU newborns. Our results suggest that strategies to reduce chronic IA in PWH are possible options for improving vaccine immunogenicity.

Introduction

Each year, an estimated 1.3 million women living with human immunodeficiency virus (HIV) become pregnant.[1] Antiretroviral therapy (ART) has significantly reduced mother-to-child transmission of the virus. As a result, the number of HIV-exposed uninfected (HEU) infants is increasing. HEU are at higher risk of morbidity and mortality from infectious diseases than HIV unexposed (HUU) infants.[2,3] Among those infections, pertussis (whooping cough) is a highly contagious respiratory disease reemerging in recent years despite high immunization coverage.[4] The greatest risk of severe disease is reported in infants from birth to 2 months of age, when pertussis vaccination is usually initiated.[5] A recent meta-analysis of studies performed in low- and middle-income countries found an increased probability of *Bordetella pertussis* infection, as well as higher incidence, and greater rates of pertussis-related hospitalization and death in HEU compared with HUU infants.[6]

Pertussis immunization during pregnancy with tetanus diphtheria acellular pertussis (Tdap) vaccine is an effective strategy recommended in a growing number of countries to tackle the temporal immunity gap in young infants.[7–10] Vaccine-induced pertussis-specific IgG are transferred to the fetus through the placenta, providing protection during the first months of life.[11] Maternal Tdap vaccination may be particularly beneficial for HEU newborns.[12]

HIV infection is associated with persistent chronic immune activation and inflammation leading to an increased risk of developing non-acquired immunodeficiency syndrome (AIDS) comorbidities.[13,14] Untreated HIV infection correlates with both a decrease in classical memory B cells (MBC) and an increase in immature B cells, activated, and atypical MBC. Activated naive B cells contribute to hypergammaglobulinemia, whereas loss of MBC is associated with reduced levels of antigen-specific antibodies.[15] ART partially normalizes the skewed B-cell profiles, but some activation persists.[16] People living with HIV have impaired responses to vaccination, in terms of seroconversion rates and persistence of vaccine-induced antibodies for several vaccines including influenza.[7,17,18]

Despite appropriate use of ART, immune activation associated with HIV infection could impact immune responses to vaccination in pregnant women (PW) living with HIV (PWH) and may reduce the benefits of maternal immunization for HEU infants. Evidence suggests that maternal HIV infection is associated with lower levels of protective vaccine-specific antibodies and reduced efficiency of transplacental antibody transfer for immunization against influenza, tetanus, *Streptococcus pneumoniae* and group B streptococcus.[7,19,20] In addition, lower PWH:HEU transfer ratios and/or lower levels of non-vaccine antibodies in HEU have been reported for measles, varicella, tetanus, influenza, pertussis, *Streptococcus pneumoniae*, hepatitis B and respiratory syncytial virus (RSV).[7,20,21] Moreover, the association of HIV infection with changes in IgG subclasses and biophysical characteristics, including Fc glycan pattern and functionality, may also have an impact on transfer.[22–24]

Regarding pertussis, although there is no universally accepted immunological correlate of protection, the level

of anti-PT (pertussis toxin) IgG antibodies is generally considered the main correlate of protection.[25] In addition to binding and direct neutralization, which is commonly evaluated in vaccine studies, Fc-mediated functions such as complement deposition and phagocytosis by macrophages and neutrophils play an essential role in protection against *B. pertussis*.[26,27] A pertussis booster vaccination is recommended in every pregnancy to ensure adequate levels of antibodies in cord blood.[8] Importantly, changes in B-cell subsets reported in people living with HIV may also influence the quality of the vaccine response.[15] With the resurgence of pertussis infections in newborns[28] and the impaired immune development in HEU,[29] it is crucial to understand the transfer of pertussis-specific immunity in this population.

We have previously reported that pregnancy does not affect the quality of effector IgG and MBC responses to Tdap immunization and that polyfunctional IgG are efficiently transferred across the placenta.[30] We sought to characterize the impact of chronic HIV infection and associated immune activation during pregnancy on the magnitude and quality of IgG and MBC responses to pertussis and tetanus immunization by comparing humoral immune responses in PWH and HIV-uninfected PW.

Methods

Study design

Following written informed consent, PWH and HIV-uninfected PW were recruited between February 2017 and 2020 at CHU Saint-Pierre, Brussels, at the antenatal clinic. Women were immunized with the combined tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap, Boostrix®, GSK Biologicals) as recommended by Belgium NITAG.[8] Serum and peripheral blood mononuclear cells (PBMCs) were collected before, 7–10 days, and 30 days after immunization and at delivery. Cord blood was collected at birth.

Soluble inflammatory markers

Levels of sCD14, sCD163, sCD25, IP-10, IFN-γ, IL-6, IL-10, and TNF-α were quantified in serum using a custom multiplex human cytokine magnetic bead assay according to the manufacturer's instructions (Bio-Techne). IFN-γ, IL-6, IL-10, and TNF-α were quantified using high sensitivity assays. Data were acquired with a Bio-Plex 200 and analyzed with Bio-Plex Manager software (version 5, Bio-Rad Laboratories). The results were expressed as MFI and markers concentration was calculated on the basis of standard curves and expressed in ng/mL for sCD14 and sCD163 and in pg/mL for the other markers.

T-cell subsets phenotyping and activation markers

The frequency of T-cell subsets and immune activation were quantified by flow cytometry as previously described.[31] PBMCs were stained for cell surface expression and immune activation markers with the following fluorescent monoclonal antibodies: CD3 BV510, CD4 PerCP Cy5.5, CD8 AF700, CR45RO APC-H7, CCR7 PECF594, CD27 PE-Cy7, CXCR5 AF647, CXCR3 BV605, CD38 PE, HLA-DR FITC, PD-1 BV650, ICOS BV421 (BD). After surface labeling, cells were permeabilized and fixed with BD Cytofix/Cytoperm, and stained with Ki-67 BV711 for intracellular expression (BD). T-cell subsets were identified using the markers and the gating strategy depicted in the panel (Fig. 2). T-cells were defined as $CD3^+$ and discriminated into $CD8^+$ T-cell cytotoxic and $CD4^+$ T-cell helper. Among $CD4^+$ T cells, CD27 and CD45RO expressions were used to distinguish naive cells that are $CD27^+CD45RO^-$, effector cells (Teff) that are $CD27^+CD45RO^-$ and effector memory cells (TEM) that are $CD27^-CD45RO^+$. Among $CD27^+CD45RO^+$, CCR7 and CXCR5 were used to discriminate central memory cells (TCM) that are $CCR7^+CXCR5^-$, transitional memory cells (TTM) that are $CCR7^-CXCR5^-$, and pTfh cells which are $CCR7^+CXCR5$. T-cell subsets were quantified using the BD LSRFortessa cytometer and a minimum of 100,000 $CD3^+$ T-cells were acquired. Data were analyzed with FlowJo software version 10.7.1 (BD) and frequencies of T-cell subsets were expressed as a percentage of total $CD4^+$ T cells in individual subjects. Expression of CD38, HLA-DR, PD-1, ICOS, and Ki-67 was expressed as positive percentages in $CD4^+$, $CD8^+$, and pTfh cells.

B-cell subsets phenotyping

Frequencies of peripheral blood B-cell subsets were analyzed by flow cytometry as previously described.[15,32] PBMCs were stained with the following fluorescent monoclonal antibodies: CD3-V500, CD19-PECy7, CD27-BV421, CD20-BV650, CD21-PECF594, CD38-APC, CD138-PE (BD). B-cell subsets were identified using the markers and the gating strategy depicted Supplementary Figure S1. B cells were defined as $CD3^-$ and $CD19^+$ lymphocytes. Within the $CD27^+$ B cell population, CD20 and CD21 expressions were used to discriminate classical memory B cells (MBCs) $CD27^+CD20^+CD21^+$, activated MBCs $CD27^+CD20^+CD21^{low}$ and plasma cells $CD27^+CD20^-CD21^-$. Among $CD27^-$ B cells, CD20 and CD21 expressions were used to discriminate naive B cells that are $CD27^-CD20^+CD21^+$ and atypical MBCs that are $CD27^-CD20^+CD21^{low}$. B-cell subsets were quantified using the BD LSRFortessa cytometer and a minimum of 50,000 $CD3^-$ $CD19^+$ B cells were acquired. Data were analyzed with FlowJo software version 10.7.1 (BD) and frequencies of B cell subsets were expressed as the percentage of total CD19+ B cells in individual subjects.

Antigen-specific IgG

Pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and Tetanus toxoid (TT)-specific IgG antibody levels were quantified in serum using

commercial ELISA kits, calibrated with WHO standards, according to the instructions of the manufacturer (EUROMIMMUN). Antibody levels were converted into international units per milliliter (IU/mL).

Antigen-conjugated beads

MagPlex® microspheres

For multiplex assays, MagPlex® microspheres (Luminex) were activated with 50 mg/mL 1-ethyl-3-[3-dimethlyaminopropyl] carbodiimide-HC (EDC) and 50 mg/mL N-hydroxysulfosuccinimide (NHS) (ThermoFisher Scientific Pierce) for 20 min at room temperature (RT) in the dark. Microspheres were then incubated with 6.25 µg of PT, FHA, PRN (List Labs), or TT (Calbiochem®) for 2 h at RT in the dark.

FluoSpheres™Neutravidin™-labeled microspheres

For flow cytometry assays, antigens were biotinylated with EZ-Link™ NHS-LC-LC-Biotin according to the manufacturer's instructions (ThermoFisher Scientific Pierce). Unbound biotin was removed using Zeba™ Spin desalting columns. The biotinylated antigens were then incubated with FluoSpheres™Neutravidin™-labeled microspheres, 1.0 µm, yellow-green fluorescent for 2 h at 37 °C in the dark.

Fc-mediated antibody effector functions

IgG were purified from sera with the Melon™Gel IgG Spin Purification Kit (ThermoFisher Scientific Pierce) following the instructions of the manufacturer. IgG were incubated with antigen-coated beads for 2 h at 37 °C.

Antibody-dependent complement deposition (ADCD)

ADCD assay was used as previously described and adapted to the study.[33] Briefly, beads previously incubated with purified serum IgG were first incubated with human complement (Sigma–Aldrich) for 30 min at 37 °C. Biotinylated Monoclonal Anti-Human C3d (Quidel) and streptavidin-RPE (Agilent) were added sequentially for 30 min. Data were acquired with a Bio-Plex 200 and analyzed with the Bio-Plex Manager software (version 5, Bio-Rad Laboratories). Results were expressed in MFI.

Antibody-dependent neutrophil phagocytosis (ADNP)

ADNP assay was used as previously described.[34] Beads previously incubated with purified serum IgG were added to white blood cells (WBCs) containing neutrophils for 1 h at 37 °C after the removal of red blood cells. One same healthy donor was used as the source of WBC for all donors and time points. Cells were then stained with CD66b-Pacific Blue antibody (Biolegend). Neutrophil bead internalization was quantified using the BD LSRFortessa flow cytometer and a minimum of 10,000 granulocytes were acquired. Results were analyzed with FlowJo software version 10.7.1 (BD) by gating on neutrophils ($CD66b^+$) and expressed as a phagocytic score (PS, phagoscore):

$$phagoscore = \frac{\text{gMFI}\left(\text{bead}^+\text{neutrophils}\right) \times \left(\%\text{bead}^+\text{neutrophils of total neutrophils}\right)}{10,000}$$

Antibody-dependent cellular phagocytosis (ADCP)

ADCP assay was used as previously described.[27] Beads previously incubated with purified serum IgG were incubated with THP-1 cells overnight at 37 °C. Bead uptake was quantified using the BD LSRFortessa flow cytometer and a minimum of 2000 THP-1 cells were acquired. Results were analyzed with FlowJo software version 10.7.1 (BD) by gating on THP-1 cells and expressed as:

$$phagoscore = \frac{\text{gMFI (bead}^+\text{THP–1 cells)} \times \text{ (\%bead}^+\text{THP–1 cells)}}{10^6}$$

PT-specific IgG avidity

PT-specific IgG avidity was quantified by BLI (Bio-layer interferometry) as described previously.[35] Briefly, 25 nM of PT antigen in 10 mM sodium acetate pH5.0 was loaded for 10 min onto activated AR2G sensors (Sartorius). After preventing non-specific interactions, the sensors were first immersed in PBS solution (20 mM pH 7.5) to establish a baseline time curve and then in purified IgG at different dilutions (2×, 4×, and 6×) in 20 mM PBS pH7.5 for 10 min. After IgG association, dissociation was performed by re-immersion for 10 min in PBS solution. Kinetic parameters were determined by global fitting of the association and dissociation phases of the binding curves following a 1:1 binding model. Measurements and data analysis were performed using Sartorius HTX Octet with Sartorius 12.0 software (Sartorius). Results were expressed as K_{off} [s^{-1}].

Antigen-specific memory B cells

Frequencies of antigen-specific IgG-producing memory B-cells (MBC) were measured by ELISPOT assay as previously described.[32] Five million PBMCs were stimulated with CpG ODN2006 (InvivoGen), hCD40Ligand (Cell Signaling Technology), and IL-21 (Miltenyi Biotec)

at 37 °C. After 5 days, 200,000 cells were incubated for 1 h at 37 °C in HTS filter plates (MerckMillipore) previously coated with 10 µg/mL PT, FHA, and PRN, and 5 µg/mL TT. Plates were then incubated sequentially with BiotinSPconjugated Goat anti-human IgG Fc (Jackson ImmunoResearch), and ExtrAvidin Peroxidase (Sigma–Aldrich) and 3-amino-9-ethylcarbazole (AEC, Sigma–Aldrich). Spot counting was performed using AID ELISPOT Reader with EliSpot 5.0 iSpot Software (Autoimmun Diagnostika GmbH). Results were expressed as antigen-specific IgG-producing memory B-cells per million PBMCs.

Statistics

Data presentation and statistical analyses were performed using the GraphPad Prism software (version 9.0.0). Data are presented as geometric mean with 95% confidence interval (CI) for IgG level and median with 95% CI for other assays. Wilcoxon matched-pairs signed-rank test was used for intragroup comparisons and Mann–Whitney U test for comparisons between groups. p values <0.05 were considered statistically significant. Correlations were determined using the Spearman non-parametric correlation test. Spearman's correlation strenght were interpreted as follows: r < 0.2: none, r = 0.2–0.4: fair, r = 0.4–0.6 moderate, r = 0.6–0.8: strong and r > 0.8 very strong correlation. For multivariable analysis, we used multiple linear regression. Sample size calculation was based on a 10% decrease in PT-specific antibody at delivery in PWH based on published concentrations (mean = 31.4 IU/mL, two-sided level of alpha = 0.05, beta = 0.8).[36]

Study approval

The study was conducted in accordance with the Helsinki Declaration of the World Medical Association, approved by the local ethic committee (#B076201630448), and registered on ClinicalTrials.Gov (NCT03519373). Written informed consent of all participating subjects was received prior to participation.

Role of funders

The Funders had no role in study design, data collection, data analyses, interpretation, or writing of report.

Results

Study population

The characteristics of the 40 PWH and 42 PW enrolled in the study are presented in Table 1. PWH were more likely to be from Sub-Saharan Africa than PW. Most women were vaccinated in the 2nd trimester of pregnancy in both groups, with a longer interval between immunization and delivery in PWH than in PW (18.1 vs 13.7 weeks, p = 0.002). Three PWH and five PW were lost to follow-up during the study. Cord blood (CB) was collected from 35 HEU and 30 HUU. There were no differences in term of ethnicity and age between women with a CB available and those without CB available. Most PWH initiated ART before pregnancy with a median duration of 67.5 months (95% CI: 57.5–95.8). Before vaccination, the mean CD4 count was 657 cells/mm^3 and all except two PWH had an undetectable viral load (<50 copies/mL).

Persistent immune activation in PWH on ART

Immune activation was explored at baseline by measuring serum markers of inflammation and markers of T-cell activation. Serum levels of sCD14 and IP10 were significantly higher in PWH than in PW, whereas levels of other markers were similar in both groups (Fig. 1). The pre-vaccination CD4/CD8 ratio was lower in PWH than in PW (2.8 (95% CI: 2.4–7.1) vs 3.7 (95% CI: 3.5–6.7), p = 0.03). The percentages of CD4+ T cell subsets were similar in both groups, although effector T cells (Teff) and peripheral T follicular helper (pTfh) were slightly lower in PWH. CD38 and HLA-DR expression on CD8+ T cells and CD38 expression on CD4+ T cells were higher in PWH than in PW before vaccination. In contrast, CD8+ Ki-67$^+$ T cells tended to be lower in PWH (Fig. 2). An abnormal distribution of B-cell subsets was previously reported in people living with HIV on ART.[15] At baseline, the proportion of atypical B cells was slightly higher in PWH than in PW. One month after vaccination, the latter was significantly higher in PWH whereas classical MBC were lower in PWH (Supplementary Figure S1). Of note, ICOS expression on CD8+ and CD4+ T cells, and pTfh correlated with atypical and activated MBC in PWH (data not shown). These results indicate that despite ART and an undetectable viral load in most of PWH, immune activation and inflammation persist, while the distribution of B-cell subsets is not fully restored in PWH. These alterations may therefore impact the response to maternal Tdap vaccination.

Decreased FHA and PRN-specific IgG responses in PWH

Serum IgG titers against PT, FHA (filamentous hemagglutinin), PRN (pertactin), and TT (tetanus toxoid) were similar in both groups before and one week after vaccination, (Fig. 3). FHA and PRN-specific IgG levels were significantly lower in PWH than in PW at delivery (median 180 IU/mL vs 303 IU/mL, p = 0.01 for FHA and 181 IU/mL vs 345 IU/mL, p = 0.01 for PRN in PWH and PW respectively). Similar findings were found in an analysis restricted to PWH and PW of Sub-Saharan Africa origin (data not shown). Linear mixed models comparing the evolution of antibody levels between PWH and PW between baseline and delivery adjusted for baseline antibody levels, gestational age and ethnicity were performed and are reported in Supplemental Table S1. The model was significant only for FHA (p = 0.047). Overall, our results indicate that at

Characteristics of the study population	PWH	PW	p
Women, N	40	42	
Age, median (IQR) (years)	32.6 (30.9–36.9)	32.2 (27.2–35.2)	0.099
BMI, median(IQR) (kg/m^2)	29.6 (26.6–32.3)	26.4 (23.9–29.1)	0.024
Race, n (%)			0.00062
Sub-Saharan Africa	33 (82)	15 (36)	
North Africa	3 (8)	10 (24)	
Europe	2 (5)	12 (29)	
Asia	1 (3)	0 (0)	
Latin America	1 (3)	3 (7)	
North America	0 (0)	2 (5)	
Primiparity, n (%)	12 (30)	18 (43)	
Tdap during last pregnancy, n (%)			0.0018
Yes	9 (22)	4 (9)	
No	17 (42)	20 (48)	
Unknown	2 (5)	18 (43)	
Immunization trimester, n (%)			0.12
Second (15–27 weeks)	33 (82)	31 (74)	
Third (>28 weeks)	5 (12)	11 (26)	
Unknown	2 (5)	0 (0)	
Gestational age at vaccination, median (IQR) (weeks)	19 (18–23.7)	25.5 (22–27.7)	0.00087
Vaccination-delivery time, mean (SD) (weeks)	18.1 (5.5)	13.7 (5.9)	0.0031
Gestational age at delivery, mean (SD) (weeks)	39.4 (2.5)	38.4 (2.9)	0.914
Women at delivery, N	37	37	
Mode of delivery, n (%)			0.00017
Vaginal	16 (43)	24 (65)	
Cesarean	7 (19)	13 (35)	
Unknown	14 (38)	0 (0)	
Prematurity, n (%)			0.0011
Yes	1 (2.7)	4 (11)	
No	23 (62)	32 (86)	
Unknown	13 (35)	1 (3)	
ART initiation, n (%)		NA	
Before pregnancy	37 (92)		
During pregnancy	3 (7)		
ART initiation time, n (%)		NA	
>3 years	32 (80)		
<3 years	8 (20)		
CD4 before vaccination, median (IQR) (cells/mm^3)	657 (616–713)	NA	
Viral load (copies/mL) before vaccination, n (%)		NA	
<50 (undetectable)	38 (95)		
50–200	1 (2)		
>200	1 (2)		
Undetectable viral load time, median (IQR) (months)	54 (24.3–72)	NA	

Abbreviations: PWH, pregnant women living with HIV; PW, pregnant women; BMI, body mass index; Tdap, tetanus toxoid, reduced diphtheria toxoid, acellular pertussis; ART, antiretroviral therapy; NA, not applicable.

Table 1: **Clinical characteristics of the study population.**

the time of delivery PWH had attenuated IgG responses to Tdap vaccination.

Maternal HIV infection modulates FHA and PRN-specific IgG effector functions

To assess the impact of HIV infection during pregnancy on the functional profile of antigen-specific antibodies following Tdap vaccination, three Fc-mediated antibody effector functions were explored. Antibody-dependent complement deposition (ADCD) and phagocytosis by macrophages (ADCP) and neutrophils (ADNP) of vaccine-specific IgG were significantly increased after vaccination and until delivery in both groups (Fig. 4). One week after vaccination, approximately half of the

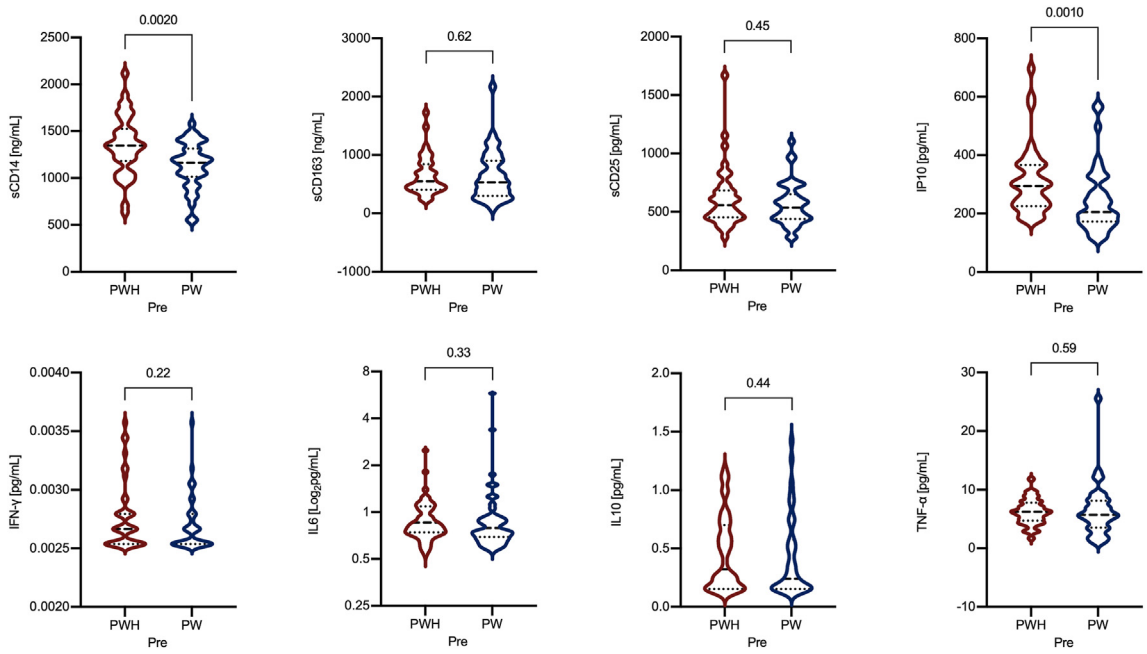

Fig. 1: **Levels of inflammatory markers in pregnant women living with HIV and HIV-uninfected pregnant women before vaccination.** Serum levels of sCD14, sCD163, sCD25, IP-10, IFN-γ, IL-6, IL-10, and TNF-α were measured by multiplex assay in 39 pregnant women living with HIV (PWH, red) and 40 HIV-uninfected pregnant women (PW, blue) before (Pre) Tdap vaccination. Results are expressed in ng/mL or pg/mL. Violin plots represent the distribution of the data with median and quartiles. Statistical significance between groups was assessed using Mann–Whitney U test.

PW had low PT-specific ADCD, which is consistent with the binding IgG results (Figs. 3 and 4A). PWH and PW had similar post-vaccination ADCP, except for PRN ADCP, which was lower in PWH than PW at delivery, in line with binding IgG results (Figs. 3 and 4B). PT ADNP was significantly higher in PWH at one month after vaccination, consistent with pre-vaccination levels. FHA ADNP was significantly lower in PWH than in PW at delivery, and PRN ADNP also tended to be lower, in accordance with the binding IgG results (Figs. 3 and 4C). Although the majority of Fc-mediated effector functions are similar between PWH and PW following Tdap immunization, HIV infection appears to partially modulate specific functions.

Lower pertussis specific IgG in cord blood of HEU infants

To evaluate the efficiency of maternal antibody transfer across the placenta, serum levels of vaccine antigen-specific IgG were measured in mothers at delivery and in cord blood. IgG levels against PT, FHA, PRN, and TT were significantly higher in CB than in maternal blood at delivery within the two groups (Fig. 5). Pertussis-specific IgG levels were significantly lower in HEU CB than in HUU CB (Fig. 5 and Supplementary Table S3). TT-specific IgG levels also tended to be lower in HEU, although the difference was not significant. Of note, PT and TT-specific IgG were transferred less efficiently in the PWH:HEU group than in the PW:HUU group, whereas transfers of FHA and PRN-specific IgG were similar in both groups, suggesting a differential impact of HIV infection on IgG transfer depending on antigen specificity. In PWH, FHA-specific IgG were transferred less efficiently than PT-specific IgG (Supplementary Figure S2). There was a higher proportion of HEU newborns with low PT-specific IgG concentration: 26% of HEU vs 13% of HUU had <30 IU/mL, while 49% of HEU vs 23% of HUU had ≤40 IU/mL (data not shown). As the two groups of mothers had different demographic features, we performed a multivariable analysis using multiple linear regression with (log-transformed) PT-specific IgG levels in cordblood as dependent variable and (log-transformed) maternal baseline PT-specific IgG, ethnicity (Sub-Saharan Africa vs non-Sub-Saharan Africa), gestational age at vaccination and HIV infection status as potential explicative. Linear regression identified 2 factors independently associated with PT-specific levels in cord blood: HIV infection and maternal baseline PT-specific IgG. Presence of maternal HIV-infection significantly decreased PT-specific in cord blood ($p = 0.004$) while maternal baseline PT-specific was positively associated with cord blood PT-specific ($p < 0.001$) Overall, these results show that HEU have lower levels of maternally acquired

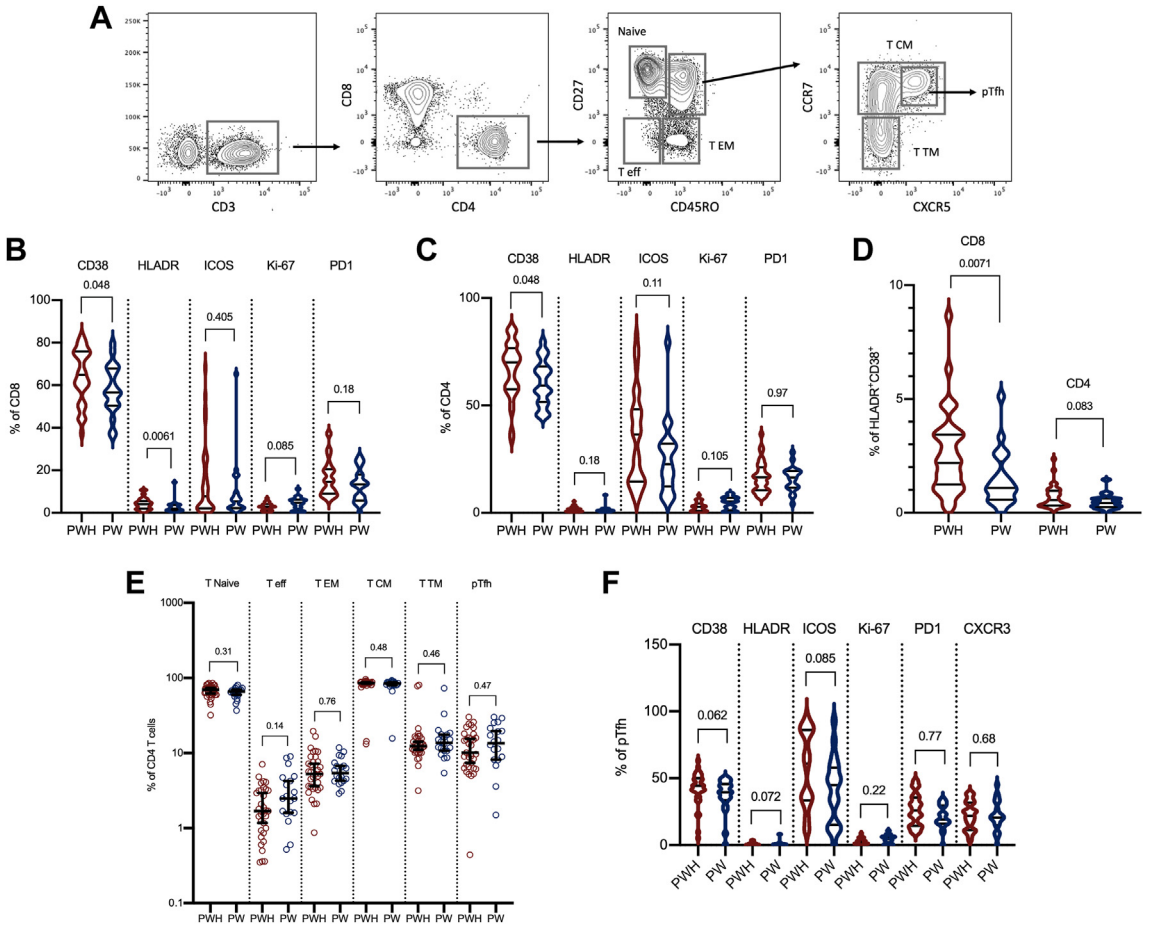

Fig. 2: **Peripheral T cell subsets and expression of immune activation markers in pregnant women living with HIV and HIV-uninfected pregnant women before Tdap vaccination.** (A) Peripheral blood T cell subsets were analyzed by flow cytometry in 33 pregnant women living with HIV (PWH, red) and 20 HIV-uninfected pregnant women (PW, blue) before (Pre) Tdap vaccination using the markers and the gating strategy depicted in the panel. Frequencies (%) of cells positive for indicated markers among (B) CD8+, (C) CD4+ and (D) HLADR + CD38+ T cells, observed in individual subjects. (E) Percentage of CD4 T cell subsets. (F) Expression of activation markers on pTfh cells. Violin plots represent the distribution of the data with median and quartiles. Lines represent the medians with 95% confidence interval (CI). T cell effector (Teff), effector memory (TEM), central memory (TCM), transitional memory (TTM), peripheral T follicular helper (pTfh). Statistical significances between groups were assessed using Mann–Whitney U test.

vaccine-specific IgG than HUU after maternal Tdap immunization and that maternal HIV-infection is an independent factor associated with lower PT-specific IgG in cord blood.

Pertussis-specific IgG Fc-mediated effector functions are reduced in cord blood of HEU infants

As observed for binding antibodies, IgG Fc-mediated effector functions were mostly increased in newborns compared with mothers in both groups (Fig. 6). Pertussis-specific ADCD was significantly lower in HEU than in HUU (Fig. 6A). However, the transfer ratios were similar between the groups, suggesting that levels in HEU mirrored those in their mothers, particularly for PT and FHA. PT ADCP was significantly lower and less transferred in HEU than in HUU, whereas PRN ADCP was significantly lower in HEU but in line with the data observed in PWH (Fig. 6B). Similarly to ADCP, FHA ADNP tended to be lower in HEU and PRN ADNP was lower in HEU (Fig. 6C). Of note, the levels and quality of pertussis-specific IgG in both groups were highly correlated (data not shown). Differences in the transfer ratios of Fc-mediated effector functions across specificities were observed (Supplementary Figure S3). In short, these data show that vertically transferred IgG Fc-mediated effector functions after maternal Tdap immunization are weaker in HEU for some antigens and likely reflects lower effector functions in PWH.

Lower PT-specific IgG avidity in HEU

Avidity is an important aspect of the quality of the immune response to pertussis vaccine.[37] As shown in Fig. 7,

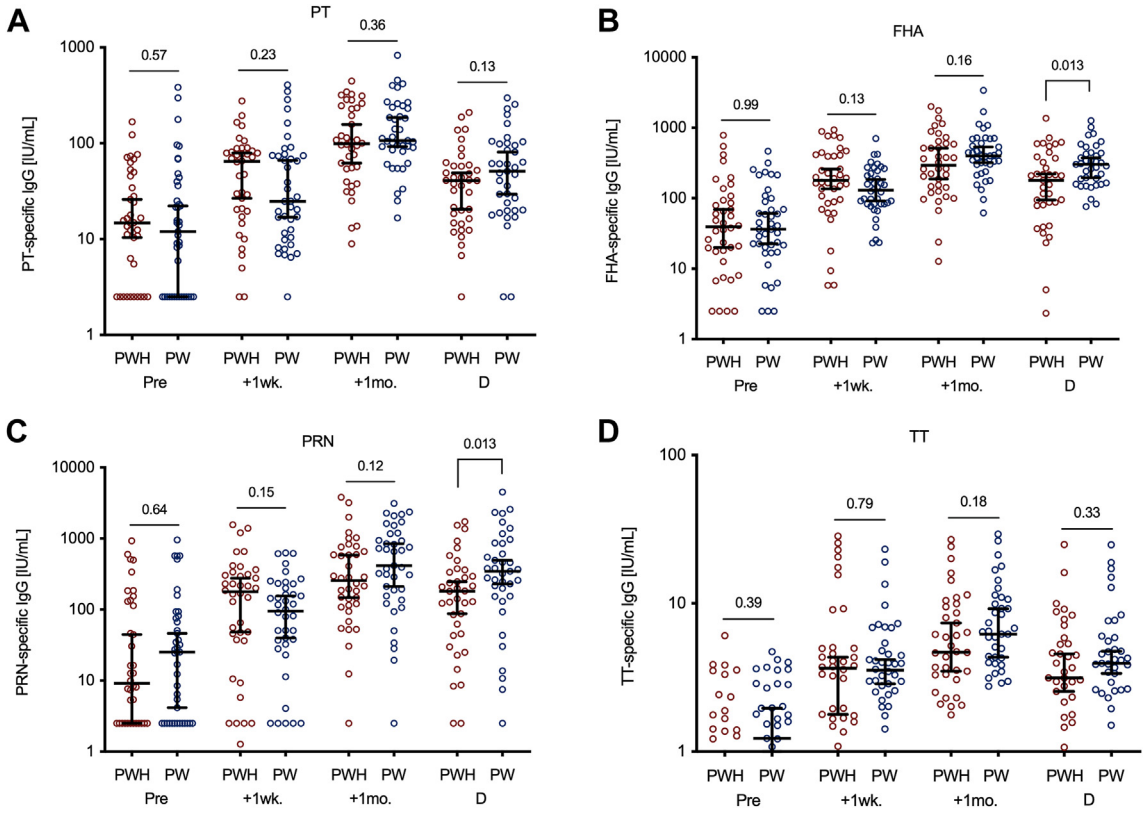

***Fig. 3:* Levels of antigen-specific IgG in pregnant women living with HIV and HIV-uninfected pregnant women before and after Tdap vaccination.** Serum levels of PT (A), FHA (B), PRN (C) and TT (D)-specific IgG (IU/mL) were measured by ELISA in 40 pregnant women living with HIV (PWH, red) and 42 HIV-uninfected pregnant women (PW, blue) before (Pre), one week (+1 wk.) and one month (+1 mo.) after Tdap immunization and, at delivery (D). Lines represent the medians with 95% confidence interval (CI) (Log_{10}). Statistical significances between groups were assessed using Mann–Whitney U test.

a significant increase in PT-specific avidity was observed in both HEU and HUU groups compared to the mothers at delivery, suggesting the preferential transfer of high-avidity IgG in the two groups, as described previously.[38] Specific avidity of PT was significantly lower in the CB from HEU than HUU. These results indicate that HEU have lower quality antibodies in terms of avidity after maternal Tdap vaccination.

Lower frequencies of FHA and PRN-specific memory B cell responses in PWH

A significant increase in the frequency of pertussis and TT-specific IgG-producing MBC was observed one month after vaccination in both groups (Fig. 8). However, the frequency of FHA and PRN-specific MBC was significantly lower in PWH than PW one month after vaccination. These results indicate that the "boosting" capacity of FHA and PRN-specific MBC is compromised in PWH. This was not the case for PT and TT, suggesting a differential impact of HIV infection on MBC responses depending on antigens. Fold-changes analyses between prevaccination and 1-months levels of vaccine-specific MBC were not different between PWH and PW (Supplemental Figure S4). Frequencies of pertussis-specific MBC in PWH after vaccination correlated with the magnitude and quality of pertussis-specific IgG in mothers and HEU (data not shown).

Decreased levels of pertussis-specific IgG are associated with persistent immune activation in PWH

We found a fair or moderate inverse correlation between the level of sCD14 in mothers before vaccination and the levels of pertussis-specific IgG in PWH at delivery and in the CB of HEU, particularly for PT and FHA (Fig. 9). PT-specific avidity and few pertussis-specific Fc-mediated effector functions were also negatively associated with maternal sCD14 levels at baseline (data not shown). Most pertussis-specific IgG levels in PWH as well as IgG levels and quality in HEU were negatively correlated with CD8+ T cells percentage, whereas they were positively correlated with CD4+ T cells percentage and CD4/CD8 ratio. Other mild inverse correlations were found, especially between pTfh cell activation

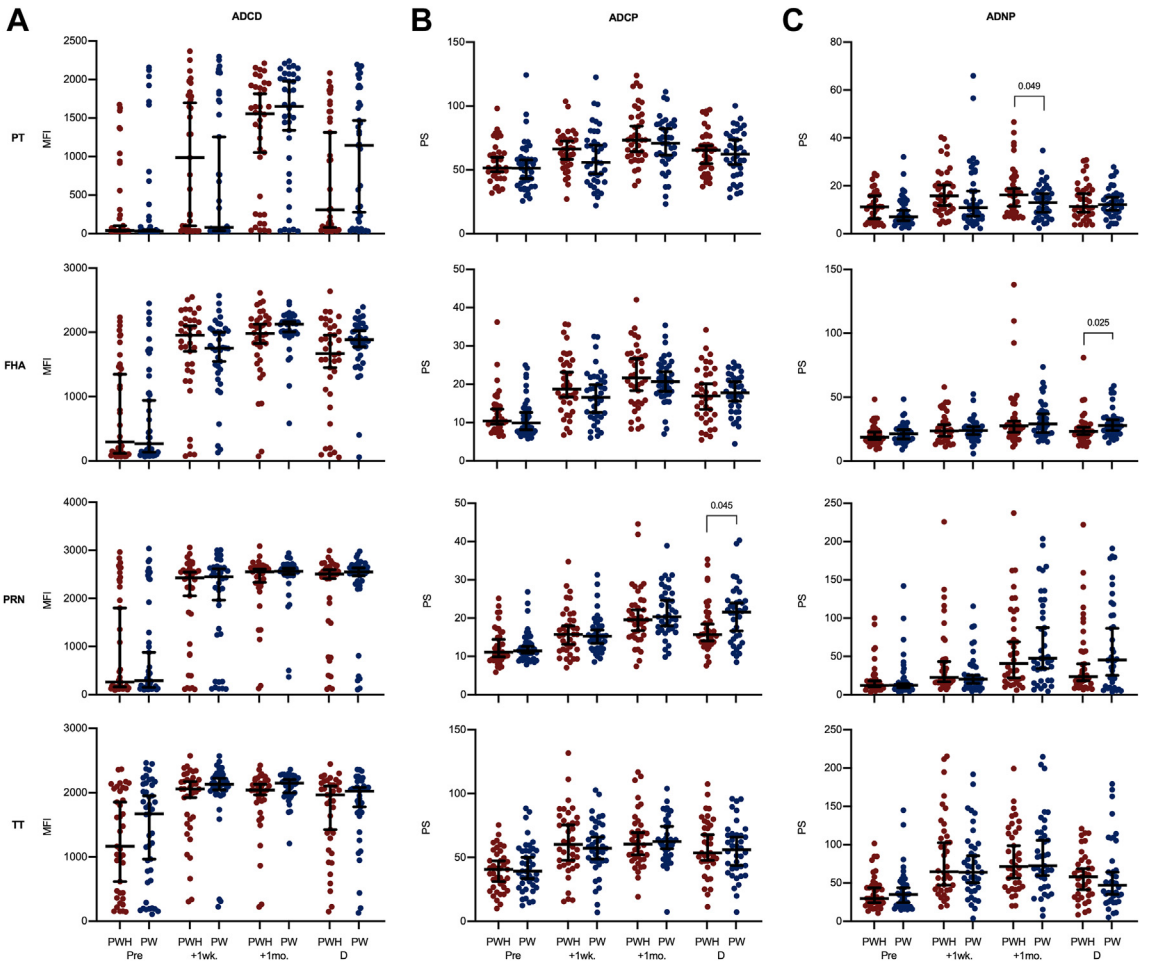

Fig. 4: **IgG Fc-dependent effector functions in pregnant women living with HIV and HIV-uninfected pregnant women before and after Tdap vaccination.** Vaccine-specific IgG Fc-dependent effector functions were measured in 40 pregnant women living with HIV (PWH, red) and 42 HIV-uninfected pregnant women (PW, blue) before (Pre), one week (+1 wk.) and one month (+1 mo.) after Tdap immunization, and at delivery (D). Antibody-dependent complement deposition (ADCD) was measured by multiplex bead array assay and is expressed as median fluorescence intensity (MFI) with 95% confidence interval (CI). (B) Antibody-dependent cellular phagocytosis (ADCP) and (C) Antibody-dependent neutrophil phagocytosis (ADNP) were measured by flow cytometry and are expressed as phagocytic score (PS) with 95% confidence interval (CI). Lines represent the medians with 95% confidence interval (CI). Statistical significances between groups were assessed using Mann–Whitney U test. p values are presented in Supplementary Table S2.

markers and anti-PRN antibodies (Table 2). Of note, all these correlations were not observed for the PW:HUU group (data not shown). Despite ART, HIV-related immune activation and CD4 alterations impact humoral responses to maternal Tdap immunization and maternal vaccine-induced pertussis-specific antibodies in their HEU infants.

Discussion

In this study, we assessed the impact of maternal HIV infection on the magnitude and quality of vaccine-specific IgG and B-cell responses to Tdap immunization. We also analyzed the quality of antibodies transferred to HEU infants.

Maternal pertussis immunization is considered the most effective strategy for reducing morbidity and mortality due to postnatal pertussis infection in infants less than 2 months of age.[39] Administration of Tdap vaccine is recommended for every pregnancy in many high-income countries.[8] A recent meta-analysis, which included only studies conducted in low- and middle-income countries, reported an increased risk of infection and pertussis-related complications in HEU compared with HUU infants,[6] emphasizing the importance of maternal Tdap vaccination among PWH.

We found an attenuated pertussis-specific IgG response following Tdap immunization in PWH compared with PW. Previous studies reported a lower seroconversion rate and rapid decrease in seroprotection

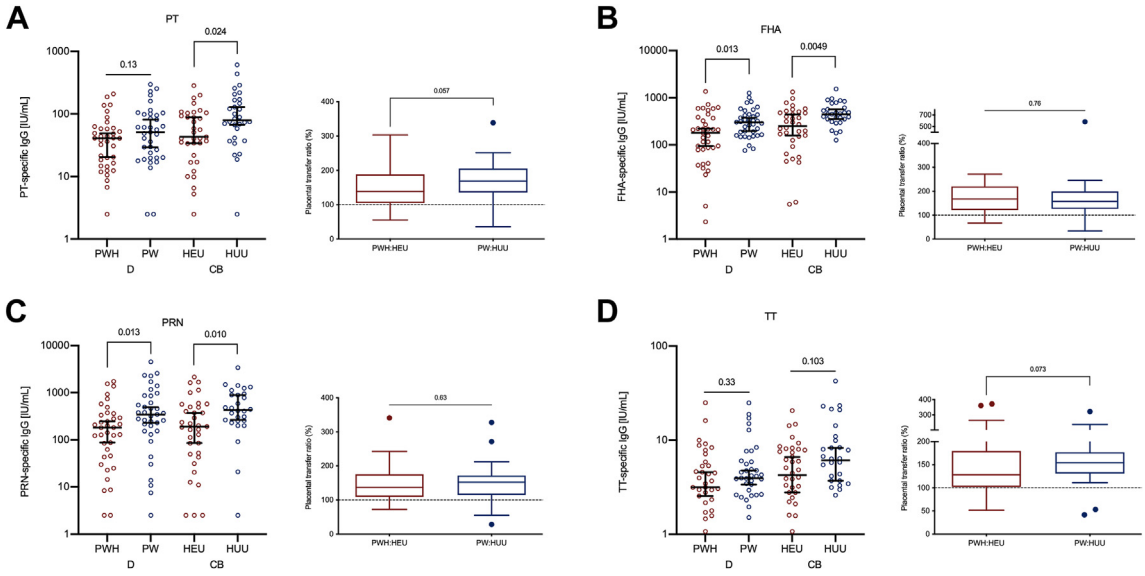

Fig. 5: **Placental transfer of antigen-specific IgG in mother:cord pairs following maternal Tdap immunization.** Serum levels of PT (A), FHA (B), PRN (C), and TT (D)-specific total IgG (IU/mL) were measured by ELISA in both 37 pregnant women living with HIV (PWH, red) and HIV-uninfected pregnant women (PW, blue) at delivery (D), as well as in the cord blood (CB) of 35 HIV-exposed uninfected (HEU) infants and 30 HIV-unexposed uninfected (HUU) after maternal Tdap immunization. Lines represent the medians with 95% confidence interval (CI) (Log_{10}). The box and whisker plots (min to max, median, quartiles) represent placental transfer ratios of antigen-specific IgG calculated as percentages of cord blood to maternal blood ratios from 34 PWH:HEU and 30 PW:HUU pairs. Dashed lines show a 100% transfer efficiency. Statistical significances between groups were assessed using Mann–Whitney test.

in people living with HIV after immunization with different types of vaccines,[7,17,18] including PWH.[19,40–45] Studies comparing vaccine responses in PWH were mostly conducted in sub-Saharan Africa, where ART uptake is lower, The differences in antibody levels following vaccination between PWH and HIV-uninfected PW were of lower magnitude as compared to previous maternal immunization studies among PWH performed in Sub-Saharan African settings with lower ART uptake. In an influenza vaccine study in South-Africa, seroconversion rate following trivalent inactivated influenza vaccine was only 40.3% among PWH as compared to 70% among HIV-uninfected PW for H1N1-specific IgG.[40] In the latter study, only 30% of PWH had CD4+ count >500/mm^3. In a group B streptococcus vaccine phase 2 trial that enrolled PWH with high and low CD4 count, GMT were consistently lower for all serotypes studies as compared to HIV-uninfected PW.[45] In line with the current study, a recent trial in South Africa also found PWH to have lower levels of antibody levels against the 4 pertussis antigens after Tdap vaccination during pregnancy.[46]

To further assess vaccine-induced antibody-dependent immunity in PWH, we performed extensive analyses of IgG Fc-dependent effector functions that are likely to be important for immunity to pertussis.[47] We found that PWH primarily developed pertussis and TT-specific antibody-dependent complement deposition as well as monocytes and neutrophils phagocytosis similarly to PW, but in contrast, they had impaired ADCP to PRN and ADNP to FHA. Differential responses of IgG Fc-dependent effector functions to influenza antigens after vaccination have been suggested in another study among PWH.[48] Overall, we showed distinct regulation of Fc effector responses across specificities, suggesting that HIV infection results in distinct humoral immunity in response to Tdap vaccination. Whether PWH might still be protected by mechanisms that could include Fc-mediated functions, independent of specific antibody titers, needs further investigation.

The efficiency of placental antibody transfer depends on multiple factors, including maternal total and specific IgG levels, placental integrity, gestational age, and biophysical characteristics of antibodies, such as IgG subclasses and Fc glycans.[49,50]

Studies on the effects of maternal HIV infection on placental function are scarce.[51] However, limited evidence suggests that maternal HIV infection is associated with reduced efficiency of transplacental antibody transfer due to placental abnormalities and hypergammaglobulinemia.[7,20] Here, we showed that the impaired response to Tdap immunization in PWH results in significantly lower levels of pertussis-specific IgG in HEU than HUU. No correlate of protection against postnatal pertussis infection has been identified

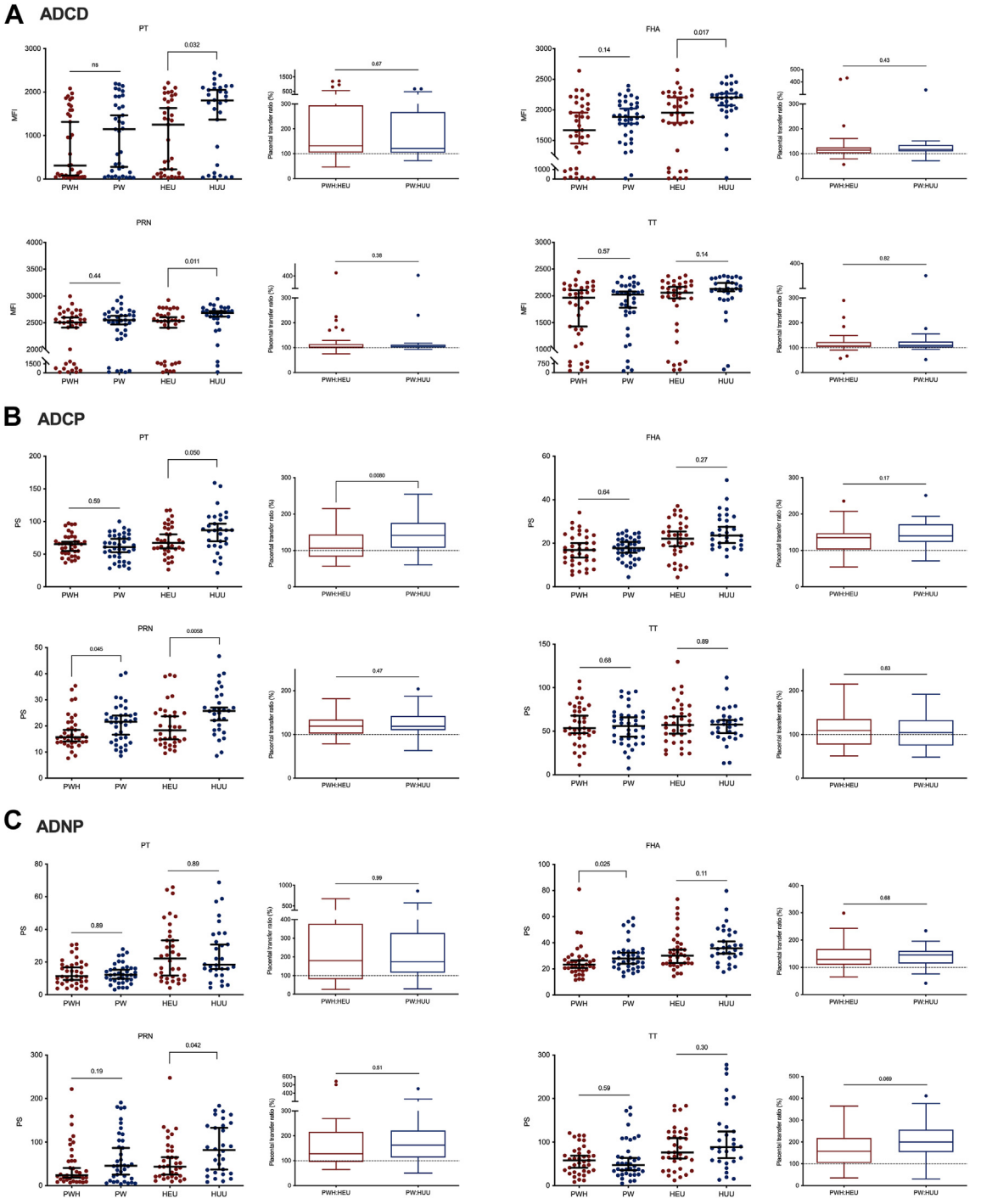

Fig. 6: **Placental transfer of antigen-specific IgG Fc-dependent effector functions in mother:cord pairs following maternal Tdap immunization.** PT, FHA, PRN, and TT-specific IgG Fc-dependent effector functions were analyzed in both 37 pregnant women living with HIV (PWH, red) and HIV-uninfected pregnant women (PW, blue) at delivery (D) and in the cord blood (CB) of 35 HIV-exposed uninfected (HEU) infants and 30 HIV-unexposed uninfected (HUU) infants after maternal Tdap immunization. (A) Antibody-dependent complement deposition (ADCD) was measured by multiplex bead array assay and is expressed as median fluorescence intensity (MFI) with 95% confidence interval (CI). (B) Antibody-dependent cellular phagocytosis (ADCP) and (C) Antibody-dependent neutrophil phagocytosis (ADNP) were measured by flow cytometry and are expressed as phagocytic score (PS) with 95% confidence interval (CI). The box and whisker plots (min to max, median, quartiles) represent placental transfer ratios of antigen-specific IgG Fc-dependent effector functions calculated as percentages of cord blood to maternal blood ratios from 34 PWH:HEU and 30 PW:HUU pairs. Dashed lines show a 100% transfer efficiency. Statistical significances between groups were assessed using Mann–Whitney U test.

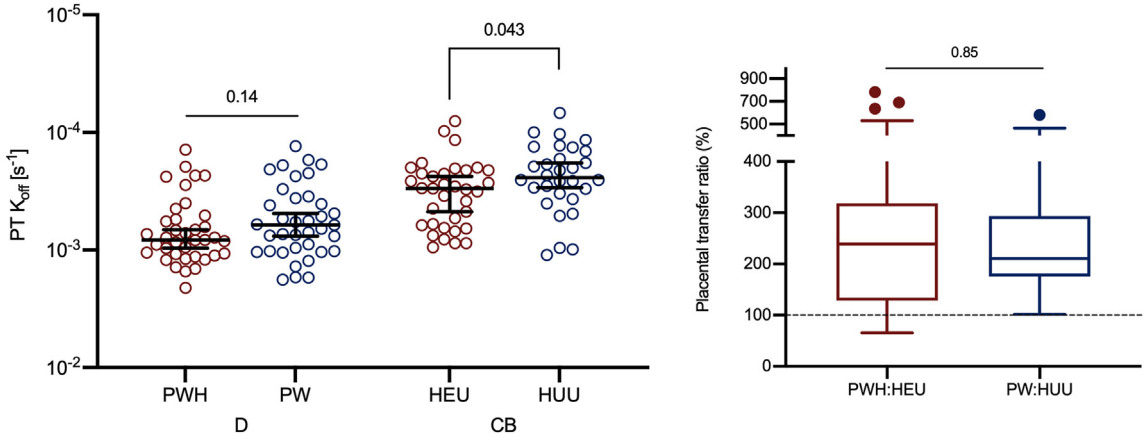

***Fig. 7*: Placental transfer of PT-specific IgG avidity in mother:cord pairs after maternal Tdap immunization.** PT-specific IgG avidity (K_{off} [s^{-1}]) was measured by BLI (Bio-layer interferometry) in both 37 pregnant women living with HIV (PWH, red) and HIV-uninfected pregnant women (PW, blue) at delivery (D), as well as in the cord blood (CB) of 35 HIV-exposed uninfected (HEU) infants and 30 HIV-unexposed uninfected (HUU) after maternal Tdap immunization. Lines represent the medians with 95% confidence interval (CI). The box and whisker plots (min to max, median, quartiles) represent placental transfer ratios of antigen-specific IgG avidity calculated as percentages of cord blood to maternal blood ratios from 34 PWH:HEU and 30 PW:HUU pairs. Dashed lines show a 100% transfer efficiency. Statistical significances between groups were assessed using Mann–Whitney U test.

to date. Different levels of evidence however strongly support an important role of anti-PT antibodies, that correlates with neutralizing anti-PT responses,[52] in the prevention of severe *B. pertussis* infection. In Denmark, where a PT-only vaccine is used, a correlation was found between one-month post-vaccination anti-PT antibodies levels and protection against severe disease.[53] An anti-PT neutralizing antibody was shown to protect against severe post-natal *B. pertussis* infection in a baboon model of infection.[54] Different thresholds, representing potentially protective levels up to infant immunization series begins, have been suggested for the concentration of anti-PT antibodies depending on its half-life.[55] Taking these into account, a greater proportion of HEU had PT-specific IgG <30 IU/mL. PT-specific IgG were transferred less efficiently in the PWH:HEU group than in the PW:HUU group, whereas the transfer ratios of FHA and PRN-specific IgG were similar between the groups. These data highlight a reduction in transplacental transfer of anti-PT antibodies, whereas the reduced levels of FHA and PRN in HEU appear to be directly due to lower antibody titers against these antigens in PWH. The mechanism of such differential impact based on antigen specificity is not yet clear but could include other biophysical characteristics of antibodies. Previous studies in both high-income countries and low- and middle-income countries found a decreased transfer ratio of non-vaccine pertussis antibodies and TT vaccine-induced IgG in PWH:HEU.[19,21,44,56,57]

The relatively few studies of PWH immunization with other vaccines, including influenza,[40] *Streptococcus pneumoniae*,[7] or group B streptococcus,[45] showed reduced transfer and/or lower levels of specific antibodies in HEU. Disruption of maternal HIV-associated antibody profiles is a key determinant of compromised neonatal immunity.[58] Here, we have shown that maternal vaccination in PWH may be insufficient to promote effective antibody transfer and protection of HEU.

As observed with binding antibodies, IgG Fc-mediated effector functions were predominantly weaker in the CB of HEU than in HUU, in a manner that differed by antigenic specificities and functions. Whether maternal HIV infection alters the quality of antibodies transferred to the newborn remains largely unexplored. However, it has been shown that the impact of HIV infection on the biophysical characteristics of maternal IgG antibodies and placental transfer could potentially alter antimicrobial immunity.[22] In South Africa, a reduction in ADCD to group B streptococcus was detected in PWH and HEU compared with the HIV-uninfected PW group.[59] In addition, PT-specific avidity was significantly lower in the CB of HEU than HUU. One study reported that early vaccination during pregnancy is associated with higher PT-specific avidity in CB.[60] In our study, PWH were vaccinated earlier than PW and avidity was significantly correlated with IA, indicating that alterations in PT-IgG avidity are related to HIV status.

Vaccine-specific MBCs are necessary for the persistence and "boostability" of humoral immunity. Tdap booster vaccination is recommended for every pregnancy to provide the newborn with sufficiently high levels of maternal antibodies.[8] We observed that PWH developed lower FHA- and PRN-specific MBC responses to Tdap vaccination than uninfected PW,

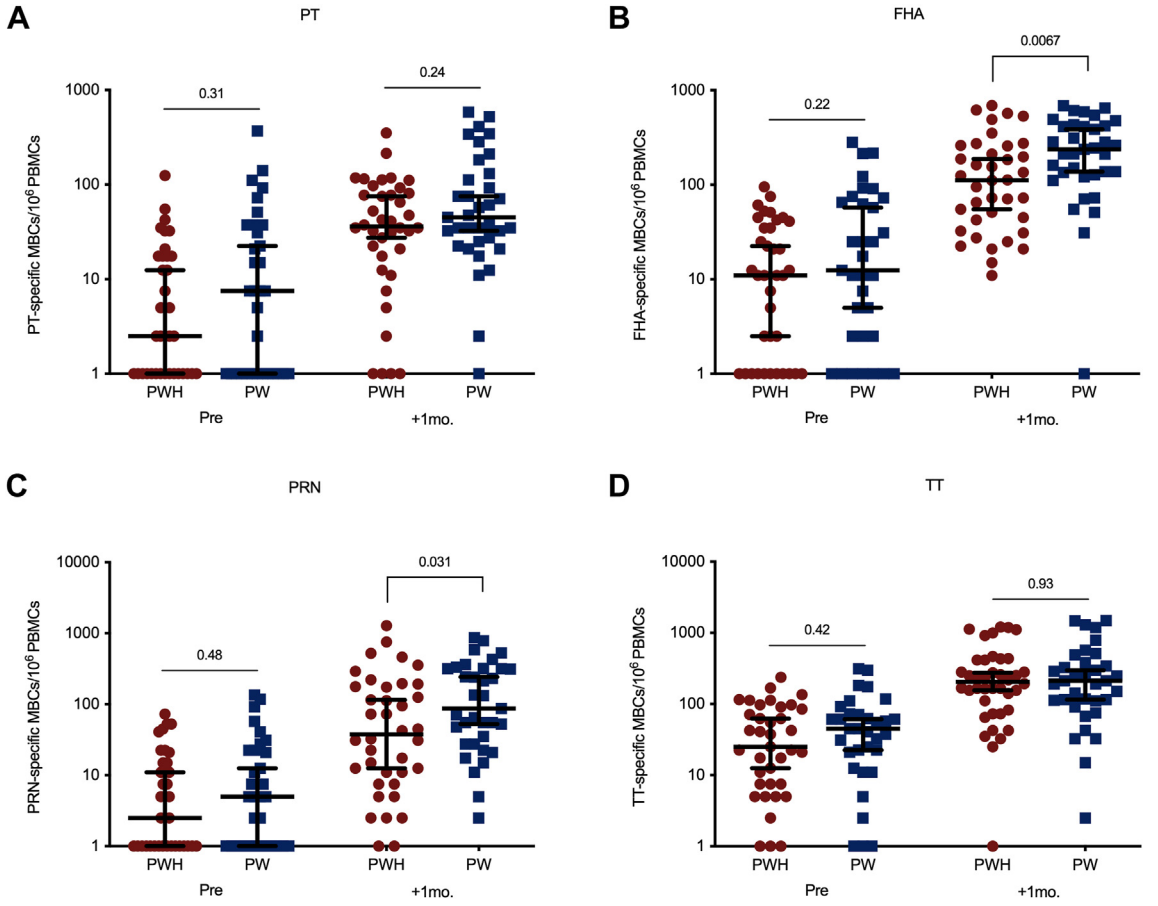

***Fig. 8:* Frequencies of antigen-specific memory B cells in pregnant women living with HIV and HIV-uninfected pregnant women before and after Tdap vaccination.** (A) PT (B) FHA (C) PRN and (D) TT-specific IgG producing memory B cells (MBCs) were measured by ELISPOT in 38 women living with HIV (PWH, red) and 35 HIV-uninfected pregnant women (PW, blue) before (Pre) and after (+1 mo.) Tdap vaccination. Off-scale values (i.e., 0) are represented on the axe. Data are expressed as medians with 95% confidence interval (CI) of the numbers of MBCs per million PBMCs (Log_{10}). Statistical significances between groups were assessed using Mann–Whitney U test.

suggesting that maternal HIV infection affects both the induction and the persistence of vaccine responses to Tdap booster vaccination. Previous research has demonstrated an impact of HIV infection on deficient MBC responses to influenza vaccination in ART-treated people living with HIV.[61,62]

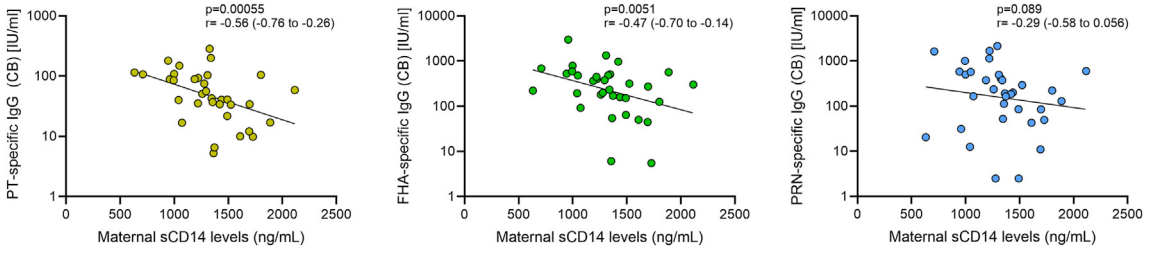

***Fig. 9:* Association between serum levels of sCD14 in pregnant women living with HIV and pertussis-specific IgG levels in HIV-exposed uninfected infants.** Simple linear regression comparing serum sCD14 levels in 35 pregnant women living with HIV (PWH) before vaccination with pertussis-specific IgG titers in the cord blood of their 35 HIV-exposed uninfected (HEU) infants after maternal Tdap immunization. sCD14 levels were measured by multiplex bead array assay and are expressed as ng/mL. Serum levels of PT, FHA and PRN-specific IgG (IU/mL) were measured by ELISA. Correlations were determined using the Spearman non-parametric correlation test.

	PWH (Delivery)						HEU (CB)					
	PT IgG		FHA IgG		PRN IgG		PT IgG		FHA IgG		PRN IgG	
	r	p	r	p	r	p	r	p	r	p	r	p
PWH (Pre)												
sCD14	−0.38 (−0.63 to −0.051)	0.021	−0.31 (−0.58 to 0.033)	0.068		0.33	−0.56 (−0.76 to −0.26)	0.00055	−0.47 (−0.70 to −0.14)	0.0051	−0.30 (−0.58 to 0.056)	0.089
sCD25		0.31	−0.31 (−0.58 to 0.030)	0.065		0.22		0.11	−0.41 (−0.66 to −0.074)	0.015		0.20
CD4/CD8 (% of CD3)	0.38 (0.0062–0.65)	0.041	0.39 (0.025–0.66)	0.032	0.50 (0.15–0.7)	0.0050	0.42 (0.049–0.69)	0.025	0.36 (−0.02 to 0.65)	0.056	0.57 (0.23–0.78)	0.0017
CD8+ (% of CD3)	−0.35 (−0.63 to 0.027)	0.06	−0.36 (−0.64 to 0.013)	0.051	−0.49 (−0.72 to −0.14)	0.0059	−0.40 (−0.68 to −0.023)	0.034	−0.34 (−0.64 to 0.043)	0.072	−0.57 (−0.78 to −0.24)	0.0015
ICOS (% of CD8)		0.97		0.61		0.19		0.74		0.74	−0.35 (−0.64 to 0.042)	0.071
PD-1 (% of CD8)		0.65		0.62		0.12		0.75		0.48	−0.44 (−0.70 to −0.062)	0.020
CD4+ (% of CD3)	0.43 (0.066–0.68)	0.019	0.40 (0.037–0.67)	0.027	0.52 (0.18–0.74)	0.0034	0.46 (0.094–0.71)	0.014	0.36 (−0.027 to 0.65)	0.061	0.58 (0.25–0.78)	0.0012
ICOS (% of CD4)		0.76		0.55		0.17		0.52		0.58	−0.36 (−0.65 to 0.026)	0.060
HLA-DR/CD38 (% of CD8)		0.45		0.13		0.37		0.53		0.12		0.40
HLA-DR/CD38 (% of CD4)		0.61		0.59		0.54		0.66		0.29		0.86
HLA-DR (% of pTfh)		0.29		0.46		0.30	−0.36 (−0.65 to 0.031)	0.063		0.26	−0.36 (−0.65 to 0.026)	0.060
ICOS (% of pTfh)		0.61		0.27	−0.34 (−0.62 to 0.039)	0.070		0.38		0.30	−0.48 (−0.72 to −0.11)	0.0097
Ki67 (% of pTfh)		0.201		0.14		0.17	−0.35 (−0.64 to 0.036)	0.066		0.31	−0.36 (−0.65 to 0.025)	0.059
PD-1 (% of pTfh)		0.42	−0.35 (−0.63 to 0.025)	0.059	−0.39 (−0.66 to −0.026)	0.032	−0.25 (−0.58 to 0.14)	0.19		0.10	−0.55 (−0.77 to −0.21)	0.0023

Abbreviations: PWH, pregnant women living with HIV; HEU, HIV-exposed uninfected infant; Pre, prevaccination; CB, cord blood; PT, pertussis toxin; FHA, filamentous hemagglutinin; PRN, pertactin. Correlations were determined using the Spearman non-parametric correlation test.

Table 2: **Correlation between immune activation and pertussis-specific IgG levels in the PWH:HEU group.**

To better understand the mechanisms of the attenuated response in PWH following Tdap vaccination, we performed correlations with soluble and cellular markers of chronic immune activation. The level of sCD14, which is produced by activated macrophages, was associated with a lower PT-specific IgG response to Tdap vaccine in PWH. It was also negatively correlated with level and quality of IgG antibodies in HEU, although not statistically significant for all antigens. The drivers of HIV-associated immune activation in virally suppressed individuals may include microbial translocation due to persistent virus-induced injury in gut-associated lymphoid tissue and, consequently, an increased level of circulating LPS leading to monocyte activation and sCD14 production.[13] Different studies have shown that altered antibody response to influenza vaccine in people living with HIV is associated with immune activation and inflammation.[31,63,64] The CD4/CD8 ratio, a marker of persistent inflammation and incomplete immune reconstitution under ART[65] associated with vaccine response in people living with HIV,[66–68] also correlated with lower vaccine-specific IgG response in the PWH/HEU group.

Our study has some limitations. First, PWH were slightly older than PW. However, immune activation markers classically found in chronic HIV infection and CD4+ T cells proportion correlated with immune alterations in PWH, indicating that this effect was most likely a consequence of HIV infection. Second, the proportion of women from sub-Saharan Africa was higher in PWH than in PW. Beyond ethnicity, partial or incomplete exposure to immunization program during childhood that include pertussis vaccines might impact the ability of pertussis-specific MBC to respond to the vaccine booster dose received during this study. Nevertheless, multivariable analysis considering maternal baseline IgG, timing of vaccination, ethnicity and HIV infection identified an effect of HIV infection independent of ethnicity and differences in FHA -IgG levels were also found after linear-mixed model adjusted for ethnicity, gestational age and baseline antibody levels. Third, the interval between vaccination and delivery was significantly longer in PWH. Nevertheless, it has been reported that a longer interval between maternal vaccination and delivery was associated with higher levels of pertussis-specific IgG transferred to newborns.[10,69] Fourth, we did not record history of Tdap vaccination and differential exposure to *B. pertussis* infection could impact baseline immunity; however, both groups had similar baseline levels of pertussis-specific IgG and MBC and multivariable analysis indicated an effect of HIV infection on PT-IgG levels in cord blood independent of baseline maternal PT-specific antibody. Fifth, analysis of Fc-dependent IgG effector functions was not normalized to adjust for differences in antibody response magnitude. Previous research has indeed shown that experimentally equalizing levels of binding antibody may reveal additional functional differences.[70] Finally, our study focused on Fc-dependent IgG effector functions supposed to be affected by pregnancy,[71,72] and did not include analysis of PT neutralizing antibodies that are important for immunity to pertussis.[54] However, previous studies have shown high correlations between the levels of PT binding and neutralizing antibodies,[52] highlighting the fact that neutralization may also be affected in PWH.

In conclusion, our data indicate that PWH with a long history of ART and undetectable viral load show evidence of attenuated humoral immune response to Tdap immunization, including decreased pertussis-specific IgG, in line with recent research performed in South-Africa,[46] and Fc-mediated effector functions, as well as lower frequencies of MBC. Suboptimal maternal immunity and transfer result in impaired IgG immunity to pertussis in CB, suggesting a possible basis for increased susceptibility to pertussis in HEU infants despite maternal immunization. This reduction in antibody-dependent immunity is correlated with persistent immune activation. Nevertheless, vaccination of PWH is likely beneficial and should be part of routine prenatal care as recommended in multiples countries worldwide.[12] Strategies to decrease immune activation in ART-treated PWH with undetectable viral load should be explored to improve vaccine immunogenicity in PWH.

Contributors

M.T. performed sample collection, designed and performed the experiments, analyzed and interpreted the data, and wrote the manuscript; F.W. conceived the study, supervised and discussed the work; C.W., D.G, A.M. designed and performed the experiments; Y.J. performed sample collection and designed the experiments; C.M., K.R., D.K., A.C., C.N., S.D.W. organized the clinical study and/or recruited the study subjects; M.E.A. designed the experiments and interpreted the data; A.M., N.D. conceived the study, acquired the funding, designed the experiments, supervised and discussed the work, and wrote the manuscript. M.T., A.M., and N.D. have accessed and verified the underlying data. All authors reviewed, edited, and approved the manuscript.

Data sharing statement

Data reported in this paper will be shared by the corresponding author upon reasonable request.

Declaration of interests

Y.J reports support from the F.R.S-FNRS. N.D. reports grant support from MSD, honoraria from Boerhingher Ingelheim, and support for attending meetings and/or travel from MSD, ViiV healthcare and Eumedica, all unrelated to the present work. D.K reports honoraria from MSD, ViiV, Janssens, support for attending meetings and/or travel from Gilead, Pfizer, MSD, ViiV and Janssens, participation on advisory board for MSD, all unrelated to the present work. M.A. reports grants from NIH, Bill and Melinda Gates Foundation, SD Ireland Foundation, consulting fees from Seromyx systems and support for attending meetings and/or travel from Keystone conferences, Bill and Melinda Gates.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105179.

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