ISSN: 2475-6296

Research Article

Journal of Clinical & Experimental Immunology

Antibody Responses Specific to Hepatitis B Virus Vaccine in Children Exposed *In-Utero* to Antiretroviral Therapy

Thibau F Tchouangueu[1,2], Laure B Kouitcheu Mabeku[2], Abel Lissom [1,7], Loveline N Ngu[1,8], Salomon B Tchuandom[2,9], Jules C Tchadji[1,7], Larissa Djukouo[1,8], Carole S Sake Ngane[1,10], Alfred A Ngoh[1,11], Hervé F Ouambo[1,12], Georgia Ambada[1,7], Anna Gutiérrez[3], Charles O Esimone[4] , Chae Gyu Park[5, 13], Alain Bopda Waffo[6, 14, 15] and Godwin W Nchinda[1*]

[1]*Laboratory of Vaccinology/Biobanking, CIRCB Cameroon*
[2]*Department of Biochemistry, University of Dschang, Cameroon*
[3]*Centre de santé catholique de Bikop (CSCB), Cameroon*
[4]*Department of Pharmaceutical Microbiology & Biotechnology, Nnamdi Azikiwe University, Cameroon*
[5]*Department of immunology, Yonsei University College of Medicine, South Korea*
[6]*Laboratory of Cellular Physiology and Immunology, Rockefeller University, USA*
[7]*Department of Animal Biology and Physiology, University of Yaoundé I, Cameroon*
[8]*Department of Biochemistry, University of Yaoundé I, Cameroon*
[9]*Public School of medical Laboratory Technicians, Yaoundé, Cameroon*
[10]*Department of Microbiology, University of Yaoundé I, Cameroon,*
[11]*Department of Biomedical Sciences, University of Dschang, Cameroon*
[12] *Department of Medical Laboratory Sciences, University of Buea, Cameroon*
[13]*Laboratory of Immunology, Brain Korea 21 PLUS Project for Medical Science, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul 03722, Republic of Korea*
[14]*Department of Biological Sciences, Alabama State University, USA*
[15]*Microbiology Program, Department of Biological Sciences, College of Science, Technology, Engineering and Mathematics, Alabama State University, Montgomery, AL, USA*

***Corresponding author:**
Dr. Godwin W Nchinda, Laboratory of Vaccinology/Biobanking the Chantal Biya International reference center for research on the Prevention and management of HIV/AIDS (CIRCB) BP 3077 Messa Yaounde Cameroon, Tel: +23776523909; Fax: +237222315456; E-mail: nsehleseh@gmail.com

Submitted: 03 July 2019; **Accepted**: 10 July 2019; **Published**: 19 July 2019

Abstract

The use of antiretroviral (ARV) has been one of the most effective means of preventing vertical transmission of the human immunodeficiency virus (HIV) to exposed children born of HIV infected mothers. Nevertheless, responses to childhood vaccination against Hepatitis B virus (HBV) infections remain suboptimal in HIV exposed uninfected children irrespective of maternal ARV prophylaxis. In a cross-sectional study we have assessed the impact of in-utero exposure to ARV on paediatric HBV vaccination. Anti-HBV surface antigen specific antibodies (anti-HBs abs) were measured in plasma specimens from 44 healthy children unexposed to both HIV and ARV (HU), 25 HIV-exposed uninfected children naïve to intrauterine exposure to ARV (HEU.AR$^-$), 29 ARV and HIV-exposed uninfected children during pregnancy (HEU.ARV$^+$), 50 children vertically infected with HIV but naïve to intrauterine exposure to ARV (HEI.ARV$^-$) and 22 children vertically infected with HIV with in utero exposure to ARV (HEI.ARV$^+$). The protective seroconversion rate after childhood HBV vaccination (anti-HBs ≥10 mUI/ml) among HEU.ARV$^+$ children (58%) was significantly lower relative to both HEU.ARVc$^-$ (100%, P=0.0010) and the healthy unexposed children (92 %, P=0.0069). Similarly, HEI.ARV$^+$ children also had significantly lower anti-HBs IgM antibody responses when compared to both HU (p=0.0003) and HEI.ARV$^-$ (0.0001) children respectively. Thus in-utero exposure to ARV probably contributes in reducing HBV vaccine antibody response rate in both HIV exposed uninfected and vertically infected children after childhood vaccination. Nevertheless, the overall impact of ARV was to improve anti-HBs IgG responses in HIV infected children suggesting a possible role in immune reconstitution leading to improved IgG antibody responses.

Keywords: HBV Vaccination, HIV, PMTCT, Childhood, Intrauterine Exposure to ARV, Humoral Immune Response

Abbreviations

3TC: Lamivudine, APC: antigen presenting cells, ART: antiretroviral therapy, ARV: antiretroviral, AZT: Zidovudine, CASS: Social and Health Animation Center, CHUY: Yaounde University Teaching Hospital, CIRCB: Chantal Biya International Reference Centre for Research on the Prevention and Management of HIV/AIDS, CSCB: Bikop Catholic Health Center, EDH: Efoulan District Hospital, EDTA: Ethylene Diamine Tetra-Acetic Acid, EFV: Efavirenz, ELISA: Enzyme-Linked Immunosorbent Assay, EPI: Expanded Program on Immunization, HBsAg: Hepatitis B Surface Antigen, HBV: Hepatitis B virus, HCV: Hepatitis C Virus, HEU: HIV Exposed Uninfected, HEx: HIV Exposed, HIV: Human Immunodeficiency Virus, HU: HIV Unexposed, IFN: Interferon, IgG: Immunoglobulin type G, IgM: Immunoglobulin type M, IL: Interleukin, NVP: Nevirapine, PMTCT: Prevention of Mother-to-Child Transmission, TDF: Tenofovir Disoproxil Fumarate, TNF-α: Tumour Necrosis Factor Alpha

Background

Lifelong carriers of Hepatitis B virus (HBV) have an increased risk of dying from hepatocellular carcinoma and liver cirrhosis and remain the main reservoir for continued transmission of HBV within communities [1-3]. Chronic Hepatitis B virus infection affects approximately 350 million people worldwide, half of whom acquired the infection from perinatal transmission or in early childhood [4, 5]. Sub Saharan Africa like most low- and middle-income countries bears the brunt of the infection (with ≥8% HBsAg prevalence) [6]. Perinatal transmission remains the main route of HBV infection often leading to severe long-term sequelae [7]. In sub Saharan Africa the transmission routes and geographical areas of high prevalence of Hepatitis B virus coincides with those of the Human immunodeficiency type 1 virus (HIV) infections [8]. This results to well over 90% of HIV infected people also showing biomarkers of HBV infection and the level of chronic HBV infections in HIV positive people reaching 15 % [9]. Childhood vaccination has become the most effective way of preventing and reducing the spread thereby cutting down the risk of chronic HBV which can be greater than 90% in infants infected early on in life.

The introduction of the prevention of the mother-to-child transmission of HIV (PMTCT) has drastically reduced the mother to child transmission rate of HIV from between 14–48% to less than 3% in both high countries and resource low income countries [10-15]. Consequently, the majority of infants born of HIV-infected mother remain uninfected even following breastfeeding. This is mainly because prophylactic antiretroviral therapy (ART) significantly diminishes mother to child transmission of HIV [16, 17]. Nevertheless prophylactic antiretroviral exposes neonates both to HIV and ARV which can lead to negative clinical consequences even among uninfected children [18]. Therefore HIV exposed uninfected (HEU) children are known to show greater susceptibility to infectious diseases leading to increased morbidity and mortality compared to children born of uninfected mother [18, 19]. Morbidity includes greater rates of hospitalization, severity of infections and increased risk of contracting infections [20-25].

There is paucity of information on the impact of *in-utero* ARV exposure on hepatitis B vaccine response rates, as well as HBsAg specific IgG subclass distribution in HIV exposed children from Sub-Saharan Africa. Therefore, we determined HBV vaccine specific antibody responses in children vertically exposed to HIV with and without intrauterine exposure to ARV.

Methods

Study Area

This multicentric cross-sectional study was conducted in five health centres of Cameroon including the Yaoundé University Teaching Hospital (CHUY), Efoulan District Hospital (EDH), Bikop Catholic Health Centre (CSCB), Social and Health Animation Centre (CASS); and the Chantal Biya International Reference Centre for Research on the Prevention and Management of HIV/AIDS (CIRCB). The University Teaching Hospital is a tertiary hospital in the capital city Yaoundé, receiving a wide variety of patients from all over Cameroon. The other selected health centres are secondary health facilities within and around Yaounde, which provides health facilities to patients from rural and urban settings.

Study Population

Children aged 4 months to 5 years old, attending the selected health centres fulfilling the eligibility criteria of the study were recruited. Participants were selected using a consecutive sampling technique and written informed consent or assent was obtained from all parents or legal guardians of participants. Children were considered as having completed HBV vaccination when they must have received all the three doses of vaccine in accordance with the national immunization schedule (at 6, 10 and 14 weeks of age). Paediatric HBV vaccination was confirmed through vaccination records in the children's immunization card. Were excluded children whose parents or legal guardians refused to give assent, those who provided incomplete data and those with incomplete paediatric HBV vaccination records? We equally excluded children with positive test of any prevalent endemic infections including HBV, hepatitis C virus (HCV), Dengue virus infection and Malaria.

Variables

The socio-demographic parameters (age, sex) were requested for both children and mothers in a structured questionnaire. Data on the period during which children were exposedintrauterine to ARV (before pregnancy, during the first, the second or the third trimester of pregnancy), as well as the ART regimen. Three classes of ART regimes were considered including R1 (Tenofovir Disproxil Fumarate-Lamivudine-Efavirenz); R2 (Zidovudine-Lamivudine-Efavirenz) and R3 (Tenofovir Disoproxil Fumarate- Lamivudine-Nevirapine). Available data on the history of tuberculosis, pneucytosis, pneupathy, hepatitis B and C and toxoplasmosis infection of children were obtained from the medical records unit and children's immunization cards.

Absolute numbers of helper $CD4^+$ T cells were determined for all participants as previously reported, Malaria, HBV and HCV diagnosis were also performed for all subjects as well as Dengue Ag-IgG/IgM antibodies detection and differentiation. Children with a positive test to any of these infections were excluded. Participants were then divided into three groups, HIV unexposed, uninfected children (HU group) taken as control group, HIV vertically exposed and uninfected children from mothers free of antenatal ARV treatment ($HEU.ARV^-$ group) and those from mothers with antenatal ARV treatment ($HEU.ARV^+$ group), then HIV exposed infected children born of antiretroviral naïve mothers (HEI.ARV-

group) as well as those born of antiretroviral treated mothers (HEI. ARV+ group). Each group of children were divided according to their age in 3 ranges such as 4-12, 13-24 and 25-60 months old.

In all volunteer participants, the plasma levels of HBsAg specific antibodies as well as anti-HBs antibodies titre were determined.

Sample collection

Blood collection was done at 4 weeks after the last HBV vaccine dose. About three millilitres of peripheral blood was collected from each participant in ethylene diamine tetra-acetic acid (EDTA)-containing tubes. The samples were then transported on ice to the CIRCB for processing. Prior to plasma separation and storage, samples were tested for malaria, hepatitis B, and hepatitis C and dengue virus infections.

Sample Analysis

Malaria, HBV, HCV and Dengue diagnosis

Except for malaria and helper CD4+ T cell counts, plasma samples were used for all serological analysis. Malaria diagnosis was done using an SD BIOLINE® point of care kit (Giheung, Republic of Korea). SD BIOLINE® HBsAg and anti-HCV immunochromatographic tests were used for the diagnosis of HBV and HCV, respectively. The CTK® *OnSite* (San Diego, USA) Duo Dengue Ag-IgG/IgM rapid test was used for the simultaneous detection and differentiation of DENV specific IgM and IgG antibodies as well as NS1 antigen in samples.

CD4+ T cells numeration

Absolute numbers of helper CD4+ T cells were determined in fresh whole blood by BD multi test CD3/CD8/CD45/CD4 and TruCount tubes (BD Biosciences, San Jose, USA) according to the manufacturer's instructions.

Detection of plasma levels of HBsAg specific antibodies

Quantification of Anti-HBs in participants' plasma was done using a bioelisa anti-HBs Kit (Biokit S.A, Barcellona, Spain) according to the manufacturer's instructions. Briefly, 100 µl/well of each participant's plasma was added into an ELISA plate pre-coated with highly purified HBsAg. After which plates were incubated at 37˚C for one hour before washing 4 times with washing buffer provided with the kit. Next, 100 µl/well of HBsAg conjugated to horse radish peroxidase (HRP) was added and incubated at 37˚C for 30 minutes. Following a 4x washing, 100 µl /well of substrate was then added and incubated at room temperature for 30 minutes. The reaction was stopped with sulfuric acid (stop solution, 100 µl per well) and the OD were read at 450 nm. The concentration of anti-HBs for each participant was calculated from a standard curve generated according to the manufacturer's instructions. Anti-HBs were categorized as i) no response, ii) < 10 mIU/ml and iii) > 10 mIU/ml and the concentration equal or greater than 10 mIU/ml was taken as the protective anti-HBs seroconversion [25-43].

Quantitative determination of anti-HBs antibodies

Plasma samples were analysed for HBsAg specific antibody responses using an optimized in-house ELISA protocol previously described [44]. Briefly, 96-wells flat-bottomed high binding Costar® assay plates (CORNING, USA) were coated either with recombinant HBsAg (IMMUNODX Woburn, MA, USA) dissolved in PBS (50 ng/well) and incubated at 4°C overnight. The following day, plates were washed three times with PBST (PBS with 0.05 % Tween-20) and blocked either with 3% BSA (Carl ROTH, Karlsruhe, Germany) for one hour at 37°C. After an additional washing step, 100 µl/well of plasma diluted (1:500) in PBS was added into corresponding wells in triplicate and incubated for two hours at 37°C.

Next, the plates are washed five times as described above. Then 100µl of horseradish peroxidase (HRP)-conjugated anti-human IgG (1:2000) and horseradish peroxidase (HRP)-conjugated anti-human IgG1, IgG2, IgG3, IgG4, or IgM (1:4000) antibodies were added, and the plates were incubated for 1h at 37°C. Antibody isotypes and subclasses used in this study were mouse anti-human IgG Fc (clone JDC-10), mouse anti-human IgM (clone UHB), mouse anti-human IgG1 Fc (clone HP6001), mouse anti-human IgG2 Fc (clone 31-7-4), mouse anti-human IgG3 Hinge (clone HP6050) and mouse anti-human IgG4 pFc' (clone HP6023), all purchased from Southern Biotech® (Birmingham, USA). Plates were then washed five times and 100µl of 2, 2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) or ABTS one component HRP substrate was added to each well and incubated in the dark for 30 min. The enzyme reaction was stopped by adding a stop solution (Southern Biotech, Birmingham, USA). The optical densities (OD) were read at 405nm using a multiskan Fc Elisa microplate reader (Thermo-scientific, USA). All plasma was tested in triplicate and the mean OD values were determined after normalization with background values derived from wells treated with validated anti-HBs plasma samples.

Ethical Approval

This study was approved by the Cameroon National Ethical Committee under protocol number 2014/07/474/CE/CNERSH/SP. All parents or legal guardians gave a written informed consent. Children that tested positive for any of prevalent endemic diseases were excluded from the study and recommended to qualified paediatrician for clinical follow up.

Statistical Analysis

Continuous variables from children's characteristics and antibody response profiles were described as medians and interquartile ranges (IQR) and categorical variables were presented as percentages or proportions. Comparison between groups was made using the non-parametric Mann-Whitney *U* test and Kruskal Wallis test with Dunn's test to compare the antibodies responses. Chi square or Fischer exact tests were used for categorical variables as appropriate. The level of statistical significance was set at $p < 0.05$. Statistical analysis was performed using Graph Pad Prism version 6.0 software.

Results

Study population characteristics

A total of 205 children were enrolled in this study, among which 35 were excluded; 1 for post-natal infection, 14 for Malaria and Dengue positive, 4 for inappropriate plasma sample quantity and quality, 7 for high anti-HBc titres and 9 participants for incomplete data. The remaining 170 children were divided as follows; 44 HU children (control group), 25 HEU.ARV - children, 29 HEU.ARV+ children, 50 HEI.ARV - children and 22 HEI.ARV + children. The frequencies, median (interquartile range, IQR) age, median (IQR) CD4 counts and Mean CD4/CD8 ratio of female and male subjects of each of the subgroups were illustrated in the table 1.

Hepatitis B surface antigen vaccine response rate and anti-HBs specific antibody responses

The protective anti-HBs seroconversion (anti-HBs ≥10 mUI/ml) rate among HEU.ARV+ (58%) was significantly lower than HEU.

ARV-and their unexposed peers (92 %) (100%) children (P=0.0010 and P=0.0069, respectively) (Figure 1A). Anti-HBs IgM responses were similar in both groups of children, while a significant drop in anti-HBs IgG specific response was noticed in HEU.ARV^{+} children as compared with the HU (P=0.0015) and HEU.ARV $^{-}$ (P=0.0169) (Figure 1B). Protective anti-HBs response rate in HEI.ARV- (46%) and HEI.ARV $^{+}$ (64%) were reduced (P=0.0001 and P=0.0303, respectively) compared with the control counterparts (Figure 1C). Moreover, there was a significant decreased anti-HBs specific IgM antibody responses among HEI.ARV $^{+}$ children than the HEI.ARV^{-} (P<0.0001) and HU (P=0.0003). HBsAg specific IgG responses in both HEI.ARV $^{-}$ and HEI.ARV $^{+}$ were lower than in the control HU children (P=0.0002 and P<0.0001). Then the level of anti-HBs IgG response in the HEI.ARV $^{-}$ group significantly reduced (P=0.0241) relative to the HEI.ARV $^{+}$ group (Figure 1D).

Table 1: Study population characteristics

Children variables	Gender	Participant n (%)	Median age (IQR), in months	Median CD4 Count (IQR) in cells/ml	Mean (±SD) CD4/CD8 Ratio
HU (n = 44)	Female	20 (45.5)	10.0 (7-23)	N/A	N/A
	Male	24 (54.5)	13.0 (7-24)	N/A	N/A
HEU.ARV $^{-}$ (n = 15)	Female	7 (46.67)	6 (5.75-8)	2259 (1016-3051)	1.72 ± 0.95
	Male	8 (53.3)	18 ([14-28)	2673 (2208-3271)	1.73 ± 0.95
HEU.ARV $^{+}$ (n = 19)	Female	7 (36.8)	6 (5-12)	1680 (1673-3122)	1.54 ± 1.01
	Male	12 (63.2)	6 (6-11)	1924 (1441-3562)	1.11 ± 0.49
HEI.ARV $^{-}$ (n = 50)	Female	Female	31.5 (20-49)	1885 ([1109-2940)	2.75 ± 1.16
	Male	24 (48)	48 (32.25-49)	1468 (1129-2045)	3.41 ± 0.77
HEI.ARV+ (n = 22)	Female	9 (40.9)	20 (11.5-33)	1924 ([1441-3062)	1.47 ± 0.81
	Male	13 (59.1)	21 (14.5-34.75)	1249 ([1147-2489)	1.12 ± 0.61

IQR: Interquartile range; n: number; HU: HIV unexposed and uninfected children; HEI: HIV exposed and uninfected children; HEU: HIV exposed and uninfected children; PMTCT: prevent mother-to-child

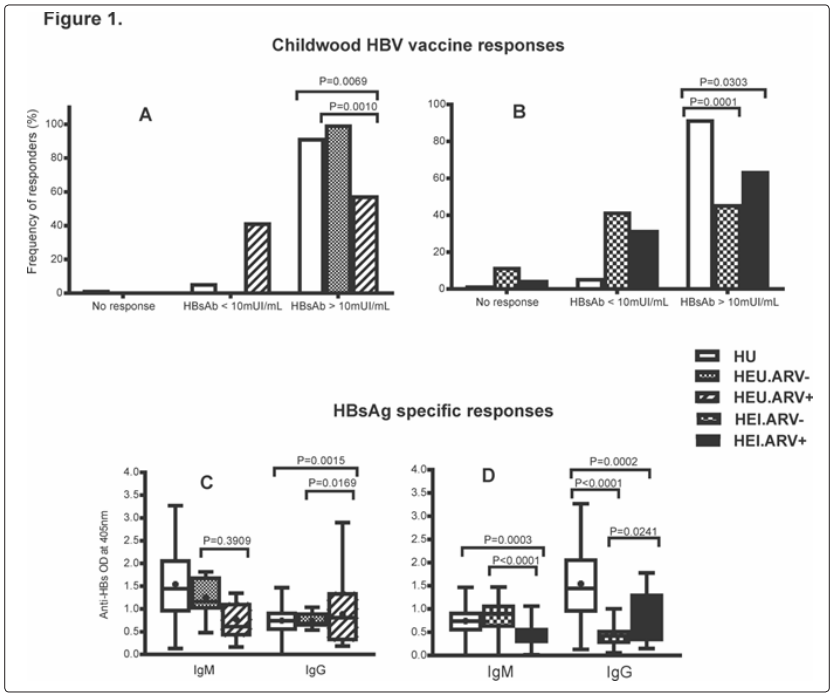

Figure 1: Hepatitis B surface antigen vaccine response rate and anti-HBs specific antibody responses in HIV unexposed (HU), HIV exposed uninfected and infected children with respect to vertical exposure to antiretroviral

In (A), is shown the proportion of children who responded to HBV vaccine in HIV exposed uninfected children, while in (B), the proportion of children who responded to HBV vaccine in HIV exposed infected children; In (C), anti-HBs specific IgM and IgG response in the similar groups of children as compared with HIV unexposed children, while in (D), anti-HBs specific IgM and IgG response in the similar groups of children as compared with HIV unexposed children. HU: HIV unexposed; HEU.ARV-: HIV-exposed uninfected children of mother who had no antiretroviral during pregnancy, HEU.ARV $^{+}$: HIV-exposed uninfected children with vertical exposure antiretroviral during pregnancy; HEI.ARV $^{-}$: HIV-exposed infected children of mother who antiretroviral naïve during pregnancy, HEI.ARV $^{+}$: HIV-exposed uninfected children vertically exposed to antiretroviral. P: p-value at 0.05 significant levels.

HBsAg specific IgG subclass antibody responses

Among HIV-exposed uninfected children, HBsAg specific IgG3 and IgG4 in the HEU.ARV^{+} were lower compared with the HU control (P=0.0190 and P<0.0001, respectively) as illustrated in Figure 2A. Anti-HBs IgG4 response was significantly lower (P<0.0001) in HEU.ARV^{+} than in their control counterpart. Moreover, HBsAg specific IgG subclass pattern of HEU.ARV^{-} and HEU.ARV^{+} children relative to control (HU) was IgG1=IgG3>IgG4=IgG2, IgG1=IgG2=IgG3=IgG4 and IgG3=IgG1=IgG4>IgG2, respectively (Figures 2B-D). In addition, there was reduced anti-HBs IgG1, IgG2, IgG3 and IgG4 response in the HEI.ARV- relative to the HU (P<0.0001). Similarly, HBsAg specific IgG3 and IgG4 antibody responses of HEI.ARV^{+} were significantly lower (P<0.0001) compared with the HU children (see Figure 3A).When examining HBsAg specific IgG subclass for each group of participants, we noticed in HU, HEI.ARV- and HEI.ARV+, patterns of IgG3=IgG1=IgG4>IgG2, IgG3=IgG2>IgG1>IgG2 and IgG1=IgG3=IgG2>IgG4, respectively (Figures 3B-D).

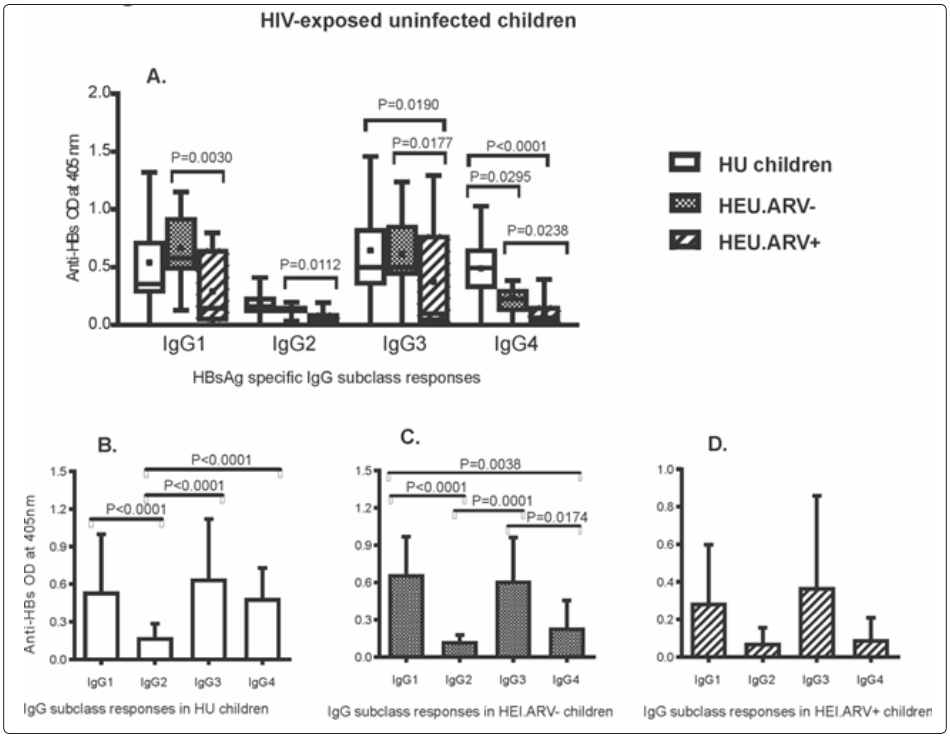

Figure 2: Hepatitis B surface antigen specific IgG subclass antibody responses in HIV unexposed (HU), HIV exposed uninfected children with respect to vertical exposure to antiretroviral

In (A), is shown the anti-HBs specific IgG subclass response among HEU children compare with the unexposed; Profile of hepatitis B surface antigen specific IgG subclass antibody responses in (B) HUx, (C) HEU.ARV^{-} (D) HEU.ARV^{+}.HU: HIV unexposed; HEU.ARV^{-}: HIV-exposed uninfected children of mother who had no antiretroviral during pregnancy, HEU.ARV^{+}: HIV-exposed uninfected children with vertical exposure antiretroviral during pregnancy; P: p-value at 0.05 significant level.

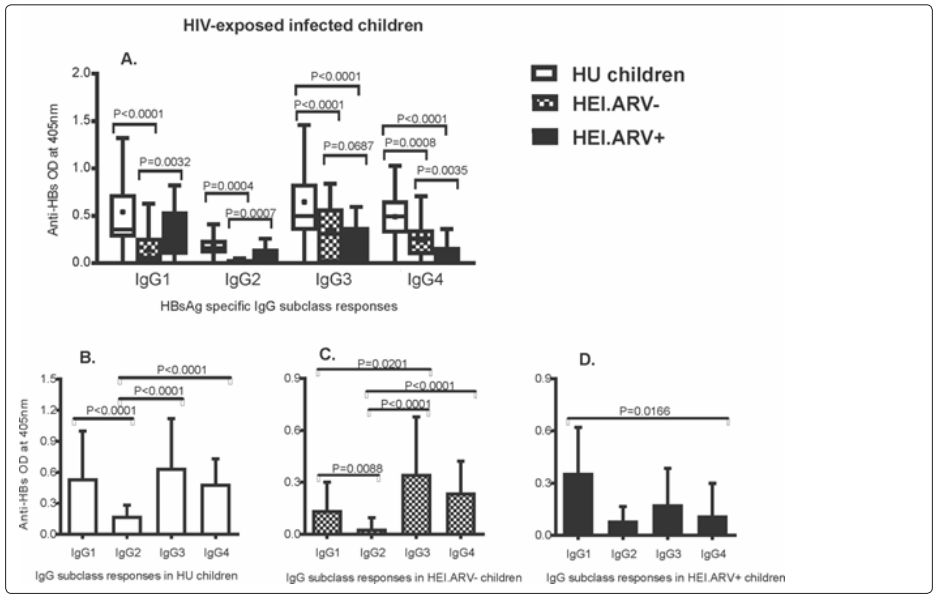

Figure 3: Hepatitis B surface antigen specific IgG subclass antibody responses in HIV unexposed (HU), HIV exposed infected children with respect to vertical exposure to antiretroviral

In (A), is shown the anti-HBs specific IgG subclass response among HEI children compare with the unexposed; Profile of hepatitis B surface antigen specific IgG subclass antibody responses in (B) HUx, (C) HEI.ARV $^{-}$ and (D) HEI.ARV $^{+}$. Children. HU: HIV unexposed; HEI.ARV $^{-}$: HIV- exposed infected children of mother who antiretroviral naïve during pregnancy, HEI.ARV $^{+}$: HIV-exposed uninfected children vertically exposed to antiretroviral. P: p-value at 0.05 significant levels.

HBsAg specific immune response among HIV/ARV exposed children according to the period of intrauterine antiretroviral exposure

Children were subdivided according to the period of intrauterine exposure to ARV consisting prior pregnancy, during the first, the second or the third trimester of pregnancy. The effect of the ARV exposure period on Hepatitis B vaccine specific antibody responses was evaluated (Table 2).

In general, seroprotective response rates among children exposed in-utero to ARV was lower than the unexposed. Despite equivalent anti-HBs IgM responses, there was a significant reduction in IgG antibody responses among ARV exposed children relative to the period of exposure. Reduction of IgG antibody responses among ARV exposed children in the 1st, 2nd and 3rd term of pregnancy was 0.64AU (P=0.0335), 0.41AU (P=0.0486) and 0.70AU (P=0.0162) respectively, relative to the control participants (1.50AU). Regarding HBsAg specific IgG subclass responses, similar levels of HBsAg specific IgG1 responses were noticed irrespective of the ARV exposure period. However, a relative reduction in anti-HBs IgG3 response was noticed among ARV exposed children relative to the period of ARV exposure when compared with HU controls (Table 2). In this regards IgG3 subclass antibody response was highly modulated among children exposed at the 2nd and at 3rd terms (0.12AU and 0.08AU, respectively) of pregnancy respectively.

Table 2: Hepatitis B surface antigen specific antibody responses rate and IgM, IgG and IgG subclass levels in HIV vertically exposed children regarding the period of intrauterine antiretroviral exposure

	HU	Pregravid ART	1st term	2nd term	3rd term
Seroprotective response rates (%)	92a	50bc	34c	63b	63b
Anti-HBs IgM	0.744 (0.53 ; 0.95)	0.40 (0.25 ; 0.54)	0.54 (0.26 ; 0.89)	0.58 (0.54 ; 1.04)	0.50 (0.27 ; 1.05)
Anti-HBs IgG	1.50 ^{a} (0.93 ; 2.11)	1.04 ^{ab} (0.28 ; 1.78)	0.64 ^{b} (0.37 ; 1.28)	0.41^{b} (0.29 ; 0.77)	0.70 ^{b} (0.24 ; 0.92)
Anti-HBs IgG1	0.35^{a} (0.27 ; 0.72)	0.06^{b} (0 ; 0.26)	0.42^{a} (0.08 ; 0.67)	0.32^{a} (0.15 ; 0.74)	0.43^{a} (0.05 ; 0.51)
Anti-HBs IgG2	0.15 ^{ab} (0.10 ; 0.24)	0.04 ^{a} (0 ; 0.07)	0.09 ^{ab} (0.01 ; 0.15)	0.10 ^{ab} (0.01 ; 0.24)	0.06 ^{b} (0 ; 0.14)
Anti-HBs IgG3	0.50 ^{a} (0.34 ; 0.84)	0.15 ^{ab} (0.02 ; 1.08)	0.15 ^{ab} (0.03 ; 0,52)	0.02 ^{b} (0 ; 0.29)	0.08 ^{b} (0 ; 0.37)
Anti-HBs IgG4	0.49 ^{a} (0.31 ; 0.66)	0.04 ^{b} (0 ; 0.14)	0.07^{b} (0 ; 0.16)	0.12 ^{b} (0.2 ; 0.31)	0.04 ^{b} (0 ; 0.15)

ART: Antiretroviral therapy; 1st, 2nd and 3rd terms: pregnancy periods of the HIV positive mothers; [a, b, c]: the difference between figures with different letters on the same row are statistically significant at P<0.05

HBsAg specific antibody response amongst children exposed in utero to ARV relative to ARV regimen

Childhood HBV vaccine specific antibody responses was also measured in children subdivided according to ARV regimen to which they were exposed during pregnancy into R1 (TDF-3TC-EFV), R2 (AZT-3TC-NVP) and R3 (TDF-3TC-NVP) in (Table 3). Our data showed that children exposed to regimens with "TDF-3TC" containing combinations, (that is R1: TDF-3TC-EFV and R3: TDF-3TC-NVP) had lower response rate (55% and 50% respectively) relative to the control HU group (92%). Similar results were observed for HBsAg specific IgG responses among children exposed in-utero to the same antiretroviral regimens. When HBsAg specific IgG subclass responses are considered, HBsAg specific IgG1 responses were not affected by exposure to any ARV regimen in contrast to a significant reduction to HBsAg specific IgG3 antibody response among children vertically exposed to R1 (0.05AU, P=0.0001) and R2 (0.21AU, P=0.0201) regimens relative to the HU control (0.50AU).

Table 3: Hepatitis B surface antigen specific antibody responses rate and IgM, IgG and IgG subclass levels in HIV vertically exposed children regarding antiretroviral regimen

	HU children	R1	R2	R3
Seroprotective response rates (%)	92[a]	55 [b]	67 [ab]	50 [b]
Anti-HBs IgM	0.74 (0.53 ; 0.95)	0.48 (0.24 ; 1.15)	0.53 (0.34 ; 0.70)	0.95 (0.49 ; 1.27)
Anti-HBs IgG	1.50 [a] (0.93 ; 2.11)	0.56 [b] (0.28 ; 0.88)	0.83 [ab] (0.32 ; 1.37)	0.69 [b] (0.29 ; 1.41)
Anti-HBs IgG1	0.35 (0.27 ; 0.72)	0.21 (0.07 ; 0.43)	0.37 (0.06 ; 0.65)	0.25 (0 ; 0.70)
Anti-HBs IgG2	0.16 [a] (0.10 ; 0.24)	0.01 [b] (0 ; 0.09)	0.11 [ab] (0.03 ; 0.17)	0 .09 [ab] (0.02 ; 0.12)
Anti-HBs IgG3	0.50 [a] (0.34 ; 0.84)	0.05 [b] (0 ; 0.15)	0.21 [b] (0 ; 0.19)	0.13 [ab] (0.04 ; 1.12)
Anti-HBs IgG4	0.49 [a] (0.31 ; 0.66)	0.05 [b] (0.05 ; 0.11)	0.05 [b] (0 ; 0.20)	0.04 [b] (0 ; 0.64)

R1: antiretroviral regimen 1 (TDF-3TC-EFV), R2: antiretroviral regimen 2 (AZT-3TC-NVP) and R3: antiretroviral regimen 3 (TDF-3TC-NVP) to which children were exposed during pregnancy; a, b: the difference between figures with different letters on the same row are statistically significant at P<0.05.

Correlation between CD4:CD8 ratio and anti-HBs specific IgM and IgG isotypes

Scatter plots were used to determine the correlations between CD4:CD8 ratios and HBsAg specific IgM and IgG responses in HEU.ARV$^-$, HEU.ARV$^+$, HEI.ARV$^-$ and HEI.ARV$^+$ children as illustrated on Table 4. A significant inverse correlation was observed only with HBsAg specific IgG for the HEI.ARV$^-$ children (r= -0.47, P=0.005).

Table 4: Correlations between CD4/CD8 ratio and anti-HBs specific IgM and IgG responses in HIV exposed children with respect to intrauterine ARV exposure

Antibody isotypes	HEU.ARV$^-$		HEU.ARV$^+$		HEI.ARV$^-$		HEI.ARV$^+$	
	r	P-value	r	P-value	r	P-value	r	P-value
IgM	0.2	0.92	0.39	0.39	-0.10	0.596	-0.41	0.247
IgG	1	0,083	0.28	0.57	-0.47	0.005	-0.38	0.279

HEU/PMTCT -: HIV exposed uninfected children born from mothers who did not prevent mother-to-child transmission; HEU/PMTCT$^+$: HIV exposed uninfected children born from mothers who prevented mother-to-child transmission; HEI/PMTCT$^-$: HIV exposed infected children born from mothers who did not prevent mother-to-child transmission; HEI/PMTCT$^+$: HIV exposed infected children born from mothers who prevented mother-to-child transmission, r: Spearman correlation coefficient; P: p-value at 0.05 significant level.

Discussion

Several studies assessing antibody responses specific to childhood vaccination against HBV in children exposed to HIV-1, show that most of these children are capable of mounting sero-protective (>10mUI/ml) levels of HBV vaccine induced antibodies [38-40, 45, 46]. However, there is uncertainty about the durability and the effectiveness of childhood HBV vaccine induced antibody responses in HEU children. HEU children have been reported to show greater morbidity and mortality than their healthy HIV unexposed uninfected counterparts. This is probably because in utero exposure to HIV might alters the immune system of the growing foetus leading to the ensuing HEU

children showing increased susceptibility to infectious diseases.

Although prophylactic ARV is important in protecting children born of HIV infected women from contracting HIV, intrauterine exposure to ARV can result to a reduction both in the rate and level of childhood HBV vaccine specific antibody responses. Therefore, in these study HEU children with in-utero ARV exposure showed significantly lower HBV vaccine response rate and diminished levels of vaccine mediated HBsAg specific IgG antibody responses than HEU children without in-utero ARV exposure. There is paucity of information about the impact of intrauterine exposure of neonates to ARV on childhood HBV vaccine mediated antibody responses. Our findings indicate that maternal ART during PMTCT probably contributes in altering antibody responses to HBV vaccine in HEU children. The mechanism leading to this alteration in immune outcomes after childhood HBV vaccination is not clearly understood but might be due to increased apoptosis in B cells of children exposed in-utero to ARV. It has been shown that HIV exposure decreases $CD4^+$ T and B cell counts and increase B cells apoptosis in HEU children [47, 48]. High HIV-1 viremia can also induce tolerance leading to immunosuppression or the dampening of HIV-specific immune response in HEU children. Chronic immune activation and increased level of pro-inflammatory cytokines (Tumor necrosis factor-α, TNF- α) can also be a compounding factor. In addition, some direct defects in immune cells such as low thymic $CD4^+$ T cell counts and impaired $CD34^+$ progenitor cell function have been reported in NRTI-exposed HEU infants [49-52].

Our data showed that the protective anti-HBs seroconversion rate in both HIV-exposed infected children (HEI.ARV^- and HEI.ARV^+) were significantly lower than their unexposed, uninfected counterparts. Several studies have reported reduced childhood HBV vaccine mediated responses in HEI children relative to their negative counterparts [52-56]. However, childhood HBV vaccine mediated responses were significantly higher in HEI.ARV^+ children than their untreated counterparts (HEI.ARV^-). This indicates the contribution of ARV in the restoration of childhood HBV vaccine mediated immune responses among children vertically infected with HIV. This was reflected in enhance B cell maturation as HBV vaccine specific IgM antibody responses were significantly higher in HEI.ARV- children than in the HEI.ARV^+ children. HIV positive individuals have previously been demonstrated to show high IgM secreting transitional B cells than HIV negative people [57]. In individuals with history of HIV infection, the percentage of plasmablasts in the peripheral blood can be higher than 50% of circulating B cells with only few of them being specific to HIV [58]. HBsAg specific IgG antibodies with neutralizing activity such as IgG1 and IgG3 are required for complete viral clearance from the host [59]. Increased HBsAg specific IgG3 subclass antibody responses is essential for the clearance of pathogen by macrophages because IgG3 antibodies are involved in complement activation and opsonisation of invading microorganisms [60].

During PMTCT ARV drugs tend to cross the placenta resulting to unborn infants being exposed to ARV in-utero. In addition during pregnancy physiological changes coupled with pharmacokinetics elements in the mother and child can affects the bioavailability of ART [61-65]. Our data showed only a slight reduction in the protective rates after childhood HBV vaccination irrespective of the period of ARV exposure suggesting that perinatal ARV exposure might not particularly affect the overall immune response. However, HBV vaccine specific IgG3 antibody subclass levels among children with exposure at the 2nd and at 3rd trimesters were significantly lower and also IgG1 at the pre-gravid period of ARV initiation, indicating that limited intrauterine ARV exposure probably negatively affected the HBV vaccine specific anti-HBs IgG3 response. On the other hand, prolonged exposure to ARV seemed to affect instead the HBV vaccine specific IgG1 subclass antibody responses. Several previous studies have also reported congenital anomalies resulting from long term exposure to antenatal ART [66-70]. Nevertheless, no specific study has focused on the impact of long-term exposure to ARV on HBsAg vaccine specific IgG and IgG subclass antibody responses.

In this study, children vertically exposed to Tenofovir Disoproxil Fumarate-Lamivudine combination had both lower childhood vaccine response rate, as well as lower HBV vaccine specific IgG antibody responses compared with to HU children. In addition, HBV vaccine specific IgG3 antibody subclass responses in children exposed to TDF-3TC-EFV and AZT-3TC-NVP regimens were lower compared to their unexposed uninfected counterparts. Overall our findings suggest that ARV mediated impairment of childhood HBV vaccine responses in children is not dependent upon a particular ARV tri-therapy combination but each tri-therapy combination might contain individual ARV with detrimental effect on antibody responses.

The relevance of the CD4/CD8 ratio as a predictor of childhood HBV vaccine response has been previously described [71]. It is known that persistent inflammation in association with a low CD4/CD8 ratio is linked to accelerated ageing among HIV positive individuals [72, 73]. Mussini and colleagues for example suggest that the normalization of CD4/CD8 ratio after highly active antiretroviral therapy (HAART) initiation correlated with a lower risk of non-AIDS related events [74]. The current study showed a significant inverse correlation between CD4/CD8 ratio and HBV vaccine specific IgG antibody responses in HEI children born of mothers who did not received ARV during pregnancy.

There is need for sustained monitoring of children after childhood HBV vaccination to determine the long-term consequences of in utero exposure to HIV and/or ARV irrespective of HIV infection. Such studies are critical for improving sustainable vaccine response rate among these children

Funding

European and Developing Countries Clinical Trials Partnership (EDTCP), Grand Challenge Canada, and The World Academy of Sciences (TWAS) for funding this work, and all the volunteers for sample donations.

Acknowledgements

We are grateful to the medical staff of the collaborating healthcare centers who participated in this study, as well as the Chantal Biya International Reference Centre for Research on the Prevention and Management of HIV/AIDS (CIRCB) for the logistic.

We thank all the women who consented for their children to participate in this study. We wish to thank EDTCP, Grand Challenge Canada, OWSD, SIDA, and TWAS for funding this work, and all the volunteers for sample donations.

Ethics approval and consent to participate

This study was approved by the Cameroon National Ethical Committee under protocol number 2014/07/474/CE/CNERSH/SP. All parents or legal guardians gave a written informed consent.

References

1. Beasley RP, Shiao IS, Wu TC, Hwang LY (1982) Hepatoma in an HBsAg carrier--seven years after perinatal infection. J Pediatr 101: 83-84.
2. Shiraki K, Yoshihara N, Kawana T, Yasui H, Sakurai M (1977) Hepatitis B surface antigen and chronic hepatitis in infants born to asymptomatic carrier mothers. Am J Dis Child 131: 644-647.
3. Mast EE, Margolis HS, Fiore AE, Brink EW, Goldstein ST, et al. (2005) A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. MMWR Recommendations and reports 54: 1-31.
4. Lavanchy D (2005) Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. Journal of clinical virology 34: S1-S3.
5. Jonas MM (2009) Hepatitis B and pregnancy: an underestimated issue. Liver Int 29: 133-139.
6. Amponsah-Dacosta E, Lebelo RL, Rakgole JN, Burnett RJ, Selabe SG, et al. (2014) Evidence for a change in the epidemiology of hepatitis B virus infection after nearly two decades of universal hepatitis B vaccination in South Africa. J Med Virol 86: 918-924.
7. Rumi MA, Begum K, Hassan MS, Hasan SM, Azam MG, et al. (1998) Detection of hepatitis B surface antigen in pregnant women attending a public hospital for delivery: implication for vaccination strategy in Bangladesh. Am J Trop Med Hyg 59: 318-322.
8. Alter MJ (2003) Epidemiology of hepatitis B in Europe and worldwide. Journal of hepatology 39: S64-S69.
9. Alter MJ (2006) Epidemiology of viral hepatitis and HIV co-infection. Journal of hepatology 44: S6-S9.
10. John GC, Kreiss J (1996) Mother-to-child transmission of human immunodeficiency virus type 1. Epidemiol Rev 18: 149-157.
11. Burns DN, Mofenson LM (1999) Paediatric HIV-1 infection. Lancet 354: SII1-SII6.
12. Kind C, Rudin C, Siegrist CA, Wyler CA, Biedermann K, et al. (1998) Prevention of vertical HIV transmission: additive protective effect of elective Cesarean section and zidovudine prophylaxis. Swiss Neonatal HIV Study Group. AIDS 12: 205-210.
13. European Collaborative S (2005) Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. Clinical infectious diseases 40: 458-465.
14. Chasela CS, Hudgens MG, Jamieson DJ, Kayira D, Hosseinipour MC, et al. (2010) Maternal or infant antiretroviral drugs to reduce HIV-1 transmission. N Engl J Med 362: 2271-2281.
15. Shapiro RL, Hughes MD, Ogwu A, Kitch D, Lockman S, et al. (2010) Antiretroviral regimens in pregnancy and breast-feeding in Botswana. N Engl J Med 362: 2282-2294.
16. Gaillard P, Fowler MG, Dabis F, Coovadia H, Van Der Horst C, et al. (2004) Use of antiretroviral drugs to prevent HIV-1 transmission through breast-feeding: from animal studies to randomized clinical trials. J Acquir Immune Defic Syndr 35: 178-187.
17. Jackson JB, Musoke P, Fleming T, Guay LA, Bagenda D, et al. (2003) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. Lancet 362: 859-868.
18. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, et al. (2014) HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol 176: 11-22.
19. Abu-Raya B, Kollmann TR, Marchant A, MacGillivray DM (2016) The Immune System of HIV-Exposed Uninfected Infants. Front Immunol 7: 383.
20. Koyanagi A, Humphrey JH, Ntozini R, Nathoo K, Moulton LH, et al. (2011) Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in Zimbabwe before availability of highly active antiretroviral therapy. Pediatr Infect Dis J 30: 45-51.
21. Slogrove A, Reikie B, Naidoo S, De Beer C, Ho K, et al. (2012) HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. J Trop Pediatr 58: 505-508.
22. Mussi-Pinhata MM, Motta F, Freimanis-Hance L, de Souza R, Szyld E, et al. (2010) Lower respiratory tract infections among human immunodeficiency virus-exposed, uninfected infants. Int J Infect Dis 14: e176-e182.
23. McNally LM, Jeena PM, Gajee K, Thula SA, Sturm AW, et al. (2007) Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. Lancet 369: 1440-1451.
24. Fawzy A, Arpadi S, Kankasa C, Sinkala M, Mwiya M, et al. (2011) Early weaning increases diarrhea morbidity and mortality among uninfected children born to HIV-infected mothers in Zambia. J Infect Dis 203: 1222-1230.
25. Kuhn L, Thea DM, Aldrovandi GM (2007) Re: HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. J Infect Dis 195: 1728-1729.
26. Adler C, Haelterman E, Barlow P, Marchant A, Levy J, et al. (2015) Severe Infections in HIV-Exposed Uninfected Infants Born in a European Country. PLoS One 10: e0135375.
27. Mussi-Pinhata MM, Freimanis L, Yamamoto AY, Korelitz J, Pinto JA, et al. (2007) Infectious disease morbidity among young HIV-1-exposed but uninfected infants in Latin American and Caribbean countries: the National Institute of Child Health and Human Development International Site Development Initiative Perinatal Study. Pediatrics 119: e694-e704.
28. Stavnezer J, Guikema JE, Schrader CE (2008) Mechanism and regulation of class switch recombination. Annu Rev Immunol 26: 261-292.
29. Stavnezer J (1996) Immunoglobulin class switching. Curr Opin Immunol 8: 199-205.
30. Stevens TL, Bossie A, Sanders VM, Fernandez-Botran R, Coffman RL, et al. (1988) Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. Nature 334: 255-258.
31. Kawasaki Y, Suzuki J, Sakai N, Isome M, Nozawa R, et al. (2004) Evaluation of T helper-1/-2 balance on the basis of IgG subclasses and serum cytokines in children with glomerulonephritis. Am J Kidney Dis 44: 42-49.
32. Kurosaki T, Kometani K, Ise W (2015) Memory B cells. Nature

Reviews Immunology 15: 149-159.
33. Crotty S (2011) Follicular helper CD4 T cells (TFH). Annual review of immunology 29: 621-663.
34. Gelinas L, Abu-Raya B, Ruck C, Cai B, Kollmann TR (2017) Hepatitis B Virus Vaccine−Induced Cell-Mediated Immunity Correlates with Humoral Immune Response following Primary Vaccination during Infancy. Immuno Horizons 1: 45-52.
35. Barcellini W, Rizzardi GP, Borghi MO, Fain C, Lazzarin A, et al. (1994) TH1 and TH2 cytokine production by peripheral blood mononuclear cells from HIV-infected patients. AIDS 8: 757-762.
36. Williams A, Steffens F, Reinecke C, Meyer D (2013) The Th1/Th2/Th17 cytokine profile of HIV-infected individuals: a multivariate cytokinomics approach. Cytokine 61: 521-526.
37. David MC, Ha SH, Paynter S, Lau C () A systematic review and meta-analysis of management options for adults who respond poorly to hepatitis B vaccination. Vaccine 33: 6564-6569.
38. Abramczuk BM, Mazzola TN, Moreno YM, Zorzeto TQ, Quintilio W, et al. (2011) Impaired humoral response to vaccines among HIV-exposed uninfected infants. Clinical and vaccine immunology: CVI 2011 18: 1406-1409.
39. Rutstein RM, Rudy B, Codispoti C, Watson B (1994) Response to hepatitis B immunization by infants exposed to HIV. AIDS 8: 1281-1284.
40. Singh DK, Kumar R, Rai R, Maurya M, Bhargava A (2016) Immunogenicity of Hepatitis B Vaccine in HIV Exposed Uninfected Infants. Indian J Pediatr 83: 172-174.
41. Gulzar N, Diker B, Balasubramanian S, Jiang JQ, Copeland KF (2011) Human immunodeficiency virus-1 infection protects against a Tc1-to-Tc2 shift in CD8(+) T cells. Hum Immunol 72: 995-1000.
42. Borges-Almeida E, Milanez HM, Vilela MM, Cunha FG, Abramczuk BM, et al. (2011) The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. BMC infectious diseases 11: 38.
43. Ambada GN, Ntsama CE, Nji NN, Ngu LN, Sake CN, et al. (2017) Phenotypic characterization of regulatory T cells from antiretroviral-naive HIV-1-infected people. Immunology 151: 405-416.
44. Tsai TH, Huang CF, Wei JC, Ho MS, Wang L, et al. (2006) Study of IgG subclass profiles of anti-HBs in populations with different HBV infection status. Viral immunology 19: 277-284.
45. Reikie BA, Naidoo S, Ruck CE, Slogrove AL, de Beer C, et al. (2013) Antibody responses to vaccination among South African HIV-exposed and unexposed uninfected infants during the first 2 years of life. Clin Vaccine Immunol 20: 33-38.
46. Simani OE, Izu A, Violari A, Cotton MF, van Niekerk N, et al. (2014) Effect of HIV-1 exposure and antiretroviral treatment strategies in HIV-infected children on immunogenicity of vaccines during infancy. AIDS 28: 531-541.
47. Miyamoto M, Pessoa SD, Ono E, Machado DM, Salomao R, et al. (2010) Low CD4+ T-cell levels and B-cell apoptosis in vertically HIV-exposed noninfected children and adolescents. J Trop Pediatr 56: 427-432.
48. Slogrove AL, Cotton MF, Esser MM (2010) Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency. J Trop Pediatr 56: 75-81.
49. Weinhold KJ, Lyerly HK, Stanley SD, Austin AA, Matthews TJ, et al. (1989) HIV-1 GP120-mediated immune suppression and lymphocyte destruction in the absence of viral infection. J Immunol 142: 3091-3097.
50. Legrand FA, Nixon DF, Loo CP, Ono E, Chapman JM, et al. (2006) Strong HIV-1-specific T cell responses in HIV-1-exposed uninfected infants and neonates revealed after regulatory T cell removal. PLoS One 1: e102.
51. Chougnet C, Gessani S (2006) Role of gp120 in dendritic cell dysfunction in HIV infection. J Leukoc Biol 80: 994-1000.
52. Rey D, Krantz V, Partisani M, Schmitt MP, Meyer P, et al. (2000) Increasing the number of hepatitis B vaccine injections augments anti-HBs response rate in HIV-infected patients. Effects on HIV-1 viral load Vaccine 18: 1161-1165.
53. Wilson CM, Ellenberg JH, Sawyer MK, Belzer M, Crowley-Nowick PA, et al. (2001) Serologic response to hepatitis B vaccine in HIV infected and high-risk HIV uninfected adolescents in the REACH cohort. Reaching for Excellence in Adolescent Care and Health. J Adolesc Health 29: 123-129.
54. Bekele Y, Yibelta D, Bobosha K, Andargie TE, Lemma M, et al. (2017) T follicular helper cells and antibody response to Hepatitis B virus vaccine in HIV-1 infected children receiving ART. Sci Rep 7: 1-11.
55. van den Berg R, van Hoogstraten I, van Agtmael M (2009) Non-responsiveness to hepatitis B vaccination in HIV seropositive patients; possible causes and solutions. AIDS Rev 11: 157-164.
56. Tchouangueu TF, Lissom A, Ngu LN, Njambe PGD, Nji NN, et al. (20180 Vertically Acquired HIV Infection in Children Modulates Hepatitis B Surface Antigen Specifc IgG Subclass Distribution After Early Childhood Vaccination Against Hepatitis B Virus Infection. Annals of Clinical & Experimental Immunology 2: 1002-1009.
57. Colineau L, Rouers A, Yamamoto T, Xu Y, Urrutia A, et al. (2015) HIV-Infected Spleens Present Altered Follicular Helper T Cell (Tfh) Subsets and Skewed B Cell Maturation. PLoS One 10: e0140978.
58. Buckner CM, Moir S, Ho J, Wang W, Posada JG, et al. (2013) Characterization of plasma blasts in the blood of HIV-infected viremic individuals: evidence for nonspecific immune activation. J Virol 87: 5800-5811.
59. Chisari FV, Ferrari C (1995) Hepatitis B virus immunopathogenesis. Annual review of immunology 13: 29-60.
60. Hjelholt A, Christiansen G, Sorensen US, Birkelund S (2013) IgG subclass profiles in normal human sera of antibodies specific to five kinds of microbial antigens. Pathog Dis 67: 206-213.
61. Loebstein R, Lalkin A, Koren G (1997) Pharmacokinetic changes during pregnancy and their clinical relevance. Clin Pharmacokinet 33: 328-343.
62. Morgan DJ (1997) Drug disposition in mother and foetus. Clin Exp Pharmacol Physiol 24: 869-873.
63. Philipson A (1977) Pharmacokinetics of ampicillin during pregnancy. J Infect Dis 136: 370-376.
64. Krauer B, Krauer F, Hytten FE (1980) Drug disposition and pharmacokinetics in the maternal-placental-fetal unit. Pharmacol Ther 10: 301-328.
65. Rakhmanina N, Phelps BR (2012) Pharmacotherapy of pediatric HIV infection. Pediatr Clin North Am 59: 1093-1115.
66. Townsend CL, Willey BA, Cortina-Borja M, Peckham CS, Tookey PA (2009) Antiretroviral therapy and congenital abnormalities in infants born to HIV-infected women in the UK and Ireland, 1990-2007. AIDS 23: 519-524.
67. Watts DH, Li D, Handelsman E, Tilson H, Paul M, et al. (2007) Assessment of birth defects according to maternal therapy

among infants in the Women and Infants Transmission Study. J Acquir Immune Defic Syndr 44: 299-305.
68. Watts DH, Huang S, Culnane M, Kaiser KA, Scheuerle A, et al. (2011) Birth defects among a cohort of infants born to HIV-infected women on antiretroviral medication. J Perinat Med 39: 163-170.
69. Knapp KM, Brogly SB, Muenz DG, Spiegel HM, Conway DH, et al. (2012) Prevalence of congenital anomalies in infants with in utero exposure to antiretrovirals. Pediatr Infect Dis J 31: 164-170.
70. Williams PL, Crain MJ, Yildirim C, Hazra R, Van Dyke RB, et al. (2015) Congenital anomalies and in utero antiretroviral exposure in human immunodeficiency virus-exposed uninfected infants. JAMA Pediatr 169: 48-55.
71. Fuster F, Vargas JI, Jensen D, Sarmiento V, Acuna P, et al. (2016) CD4/CD8 ratio as a predictor of the response to HBV vaccination in HIV-positive patients: A prospective cohort study. Vaccine 34: 1889-1895.
72. Deeks SG (2011) HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med 62: 141-155.
73. Serrano-Villar S, Moreno S, Fuentes-Ferrer M, Sanchez-Marcos C, Avila M, et al. (2014) The CD4:CD8 ratio is associated with markers of age-associated disease in virally suppressed HIV-infected patients with immunological recovery. HIV Med 15: 40-49.
74. Mussini C, Lorenzini P, Cozzi-Lepri A, Lapadula G, Marchetti G, et al. (2015) CD4/CD8 ratio normalization and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. Lancet HIV 2: e98-e106.