SMGroup

Annals of Clinical & Experimental Immunology

Research Article

Vertically Acquired HIV Infection in Children Modulates Hepatitis B Surface Antigen Specific IgG Subclass Distribution After Early Childhood Vaccination Against Hepatitis B Virus Infection

Thibau F Tchouangueu[1,2], Abel Lissom[1,3], Loveline N Ngu[1,5], Ghislain D NjambePriso[1,3], Jules C Tchadji[1,3], Carole S Sake Ngane[1,5], Georgia Ambada[1,3], Nadesh N Nji[1], Jackson J Ndenkeh[6], Larissa Djukouo[1,5], Hippolite S Magagoum[1,3], Katia Nnomo[4], Anna Gutiérrez[7], George O Chukwuma[1,8], Charles O Esimone[1], Nelly Kamgaing[1,10], Chae Gyu Park[11,12], Alain BopdaWaffo[13,14], Laure B Kouitcheu Mabeku[2] and Godwin W Nchinda[1]*

[1]Laboratory of Vaccinology/Biobanking, International Center Reference Chantal Biya, Cameroon
[2]Department of Biochemistry, University of Dschang, Cameroon
[3]Department of Animal Biology and Physiology, University of Yaoundé I, Cameroon
[4]Department of Pharmacy, University of Douala, Cameroon
[5]Department of Biochemistry, University of Yaoundé I, Cameroon
[6]Department of Biomedical Sciences, Nnamdi Azikiwe University, Nigeria
[7]Department of Medical Lab Sciences, Nnamdi Azikiwe University, Nigeria
[8]Deoartment of virology, CSCB (Centre de santé catholique de Bikop), Cameroon,
[9]Department of Pharmaceutical Microbiology & Biotechnology, Nnamdi Azikiwe University, Cameroon
[10]Department pediatrics, University teaching hospital of Yaounde, Cameroon,
[11]Department of Biological Sciences, Alabama State University, USA
[12]Department of immunology, Yonsei University College of Medicine, South Korea
[13]Laboratory of Immunology, Yonsei University College of Medicine, Korea
[14]Laboratory of Cellular Physiology and Immunology, Rockefeller University, USA

Article Information

Received date: Aug 27, 2018
Accepted date: Sep 17, 2018
Published date: Sep 20, 2018

***Corresponding author**

Godwin W Nchinda, Laboratory of Vaccinology/Biobanking, International Center Reference Chantal Biya, Cameroon,
Email: nsehleseh@gmail.com

Keywords HBcAg, Class switch, HBsAg vaccine, HIV-1, IgG subclass, HBV vaccine

Abbreviations Antiretroviral; ART: Antiretroviral therapy; BSA: Albumin Serum Bovin; CD4: Clusters of differentiation type 4; CDC: Centers for Disease Control and Prevention; ELISA: Enzyme-Linked Immunosorbent Assay; HBV: Hepatitis B virus; HBsAg: HBcAg: Hepatitis B core antigen; HBeAg: Hepatitis B envelop antigen; Hepatitis B surface antigen; HIV: Human Immunodeficiency virus; HRP: Horseradish Peroxidase; IFN-γ: Interferon gamma; Ig: Immunoglobulin; IQR: interquartile ranges; OD: Optical Densities; PMTCT: Prevention of mother-to-child transmission of HIV; PBS: Phosphate Buffered Saline; Th: T helper cells; TNF-α: Tumor necrosis factor alpha

Abstract

Pediatric vaccination against Hepatitis B Virus (HBV) leads to the induction of immunoglobulin G (IgG) antibodies specific to hepatitis B surface antigen (anti-HBs) consisting of four IgG subclasses. In the context of potential immuno-suppression arising from neonatal infection with the Immunodeficiency virus type one (HIV) an analyses of the anti-HBs response rate together with IgG subclass profiles especially in children vertically infected with HIV is of value in measuring the efficacy of childhood HBV vaccination. In this study we investigated in 50 HIV positive and 44 negative children the HBV vaccine response rate together with the profiles of IgG subclass responses specific to hepatitis B surface (HBsAg). The protective HBV vaccine response rate (anti-HBs ≥10 mUI/ml) in the HIV infected children was 36% compared to 92 % in healthy controls. Whereas no difference was observed between the HIV positive and negative children with respect to IgM responses (P=0.99), a significant reduction in HBsAg specific IgG (P<0.0001) and IgG subclass responses (P< 0.0001 for IgG1, P<0.001 for IgG2, P<0.001 for IgG3 and P<0.0001 for IgG4) was observed in HIV-1 infected children relative to their negative counterparts. Thus there is a significant reduction not only in the prevalence of sero-protective titer (anti-HBs ≥10mUI/ml) but equally in the IgG subclass responses specific to HBsAg amongst HIV-1 infected children. Both HIV positive and negative children showed minimal detectable levels of antibodies specific to the core protein (HBcAg) indicative low exposure to HBV. This was associated with elevated plasma levels of HBsAg specific IgG4 antibody responses probably indicating repeated exposure to the antigen. Our data highlights the necessity to monitor HBV vaccine responses in HIV infected children in Cameroon in order to revaccinate non-immunized children.

Introduction

Hepatitis B virus infection (HBV) remains a major public health challenge affecting well over 400 million people worldwide [1,2]. Low and middle-income countries of Asia, Western pacific and sub-Saharan Africa bear the brunt of the disease with well over8% of their populations being hepatitis B surface antigen (HBsAg) positive [3].

In Cameroon like most sub Saharan Africa, regions of intense HBV infection coincide with areas of high prevalence of the Human immunodeficiency type one virus (HIV) thereby increasing the chances of dual HIV-HBV infections occurring. Within these region the majority of HBV infections occurs before 5 years of age through horizontal transmission from family members [4].

How to cite this article Tchouangueu TF, Lissom A, Ngu LN, Priso GDN, Tchadji JC, Ngane CSS, et al. Vertically Acquired HIV Infection in Children Modulates Hepatitis B Surface Antigen Specific IgG Subclass Distribution After Early Childhood Vaccination Against Hepatitis B Virus Infection. Ann Clin Exp Immunol. 2018; 2(1): 1002.

Childhood infection with HBV increases the likelihood of chronic HBV infection (with a rate >90%) in adulthood [5] together with persistent transmission of the virus. This is further compounded by concurrent HBV-HIV infection as it escalates the chronic HBV infection rate and also accelerates liver disease progression [6]. HBV infection of HIV positive individuals is also associated with increased HBV replication rates together with a longer than usual persistence of the HBV envelope antigen (HBeAg) thereby enhancing HBV transmission risk and pathology [6-8]. Since the combined effect of early childhood HBV and dual HIV-HBV infections significantly escalate disease progression mass immunization of newborn babies against HBV is strongly recommended irrespective of HIV infection.

Although the standard three dose HBV immunization schedules induces protective HBV specific immunity (HBsAg specific antibodies ≥10 mUI/ml) in well over 90% of immune competent children a significantly lower sero conversion rate (25-41% in children) is reported in HIV infected children [9,10]. More so little is known about the HBV vaccine mediated IgG subclass responses in HIV infected individuals. There is evidence suggesting that increasing either the HBV vaccine dose or frequency in HIV infected children could improve vaccine mediated specific immunity [11,12]. However rather than recommending different vaccination schedules for HIV infected children the Current Centers for Disease Control and Prevention (CDC) guidelines suggest instead additional vaccine cycles for HIV-infected non-responder adults but not in children [13,14]. While these strategies could potentially increase HBV vaccine specific antibody titers in HIV infected individuals little is known about the profile of anti-HBs specific IgG subclass responses associated with the vaccination of HIV infected children. Never the less assessing anti-HBs IgG subclass responses following HBV vaccination of HIV infected children could provide critical information about the maturation and functions of the vaccine induced humoral immune responses.

Following natural seroconversion IgG subclass responses specific to HBsAg have been demonstrated to be restricted mainly to neutralizing IgG1 and IgG3, with only a minor contribution from IgG2 and IgG4 [15,16]. The presence of both IgG1 and IgG3 is strongly associated with Th1 cytokines such as IL-2, IFN-γ and TNF-α which are critical in cell-mediated immunity and the induction of memory IgG responses [17]. Activation of Th1 response together with HBsAg specific IgG1 or IgG3 subclass responses is thus a critical necessity in the ultimate effective neutralization of HBV [18]. Unfortunately HIV infection in attacking and destroying CD4+ T cells probably leads to immune dysfunction and impairment of cellular and humoral responses in children which is vital in sustainable protective immunity [19]. Galina's and colleagues showed a significant association between humoral immune response in 1 and 2 years old children and IFN-γ secretions [20]. These cytokines support the differentiation of CD4+ T cells to Th1 cells and suppresses the development of Th2 cells [21]. Th1 and Th2 cells activities are tightly linked to IgG subclass switch, which is an indicator of a sequential IgG subclass maturation and somatic hyper mutation of specific B cell clones [22,23].

Several studies in assessing humoral immune response to HBV vaccine in HIV infected individuals have focused on rate, quantity and duration [24-28]. However, there is limited data about the profiles of HBsAg specific IgG subclass responses of HIV-1 infected children following childhood HBV vaccination. Here we have assessed the profiles of HBsAg specific IgG subclass responses in HIV infected children following the completion of childhood HBV vaccination in Cameroon.

Methods and Participants

Study design and participants selection

This is a multicentric cross-sectional study conducted in five health centers of Cameroon namely, the Yaoundé University Teaching Hospital (CHUY), Efoulan District Hospital (EDH), Bikop Catholic Health center (CSCB), Social and Health Animation Center (CASS); and the Chantal Biya International Reference Centre for Research on the Prevention and Management of HIV/AIDS (CIRCB), Yaoundé. 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 centers are secondary health facilities within and around Yaoundé, which provides health facilities to patients from rural and urban settings.

A total of 94 children aged 4 months to 5 years old were enrolled for this study. All together 44HIV-1 negative (control group) and50 HIV-1 infected children born of antiretroviral therapy naïve HIV-1 infected mothers were recruited for this study.

The study was conducted for a period of14 months and lasted from December 2014 to March 2016. The study participants were selected using consecutive sampling technique and written informed consent or assent was obtained from parents or legal guardians of the study participants (Children aged 4 months to 5 years who had completed the pediatric HBV vaccination as stipulated by the national program on immunization were considered eligible for the study. Pediatric HBV vaccination was confirmed through vaccination records. We excluded all children whose parents or legal guardians refused to give consent, those who provided incomplete data and those with incomplete pediatric HBV vaccination. We equally excluded children who were positive for prevalent endemic infections including HBV, HCV, Dengue virus infection and Malaria.

Sample collection

About three milliliters of peripheral blood was collected from each participant in Ethylene Diamine Tetra-Acetic Acid (EDTA)-containing tubes. All samples were stored at room temperature and processed within 2 hours of collection. To obtain plasma, samples were centrifuged at 2,000 rpm for 10 min at 4°C. The plasma fraction was harvested under sterile conditions in a bio safety II hood, aliquot in small, single-use volumes and stored at -20°C until use. All plasma obtained from participants was heat inactivated for 30 minutes at 56°C prior to ELISA assay. Prior to plasma separation and storage samples were tested for malaria, filarial, dengue, hepatitis B and hepatitis C virus infections. Blood collection was done at 4 weeks after the last HBV vaccine dose.

Sample analysis

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 immuno chromatographic 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 for all the samples. Absolute numbers of helper CD4+ T cells for HIV-1+ participants 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.

Laboratory analysis for the detection of plasma levels of HBsAg specific antibodies

Quantification of Anti-HBs in participants' plasma was done using abioelisa anti-HBs Kit (Biokit S.A, Barcellona, Spain) according to the manufacturer's instructions. Briefly100ul/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 for 4 times with wash buffer provided with the kit. Next 100 ul/well of HBsAg conjugated to Horse Radish Peroxidase (HRP) is added and incubated at 37˚C for 30 minutes. Following a 4x wash, 100ul/well of substrate is then added and incubated at room temperature for 30 minutes. The reaction is stopped with 100 ul/well of sulphuric (stop solution) and the OD determine 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 concentration in this study were categorized as i) no response, ii) < 10 mIU/ml and iii) > 10 mIU/ml. The protective anti-HBs concentration was taken to be equal or greater than 10 mIU/ml.

Quantitative determination of anti-HBs and anti-HBc antibodies

Plasma samples were analyzed for HBsAg and HBcAg specific antibody responses using an optimized in-house ELISA protocol previously described [29]. Briefly 96-wells flat-bottomed high binding Costar® assay plates (CORNING, USA) were coated either with recombinant HBsAg or HBcAg (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 or 1x Roti block (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 triplicates and incubated for two hours at 37°C.

Next the plates are washed five times as described above. Then 100µl 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 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 HRP conjugated antibodies were procured 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 substrate reaction was stopped by adding a stop solution (Southern Biotech, Birmingham, USA). The Optical Densities (OD) at 405nm were read using a multiscan 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 similarly treated.

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 pediatrician for clinical follow up.

Statistical analysis

Continuous variables from children's characteristics and antibody response profiles were described as medians and Inter Quartile Ranges (IQR) and categorical variables were presented as percentages or proportions. Comparison between groups were made using the non-parametric Mann-Whitney U test for continuous variables and 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

The study included 50 HIV-1 positive children vertically infected with HIV by women who did not participate in the national PMTCT program. A total 26 females with median age 30 months (IQR 20-49) and 24 males with a median age of 48 months (IQR 34-59) were recruited. The median (IQR) CD4+ cells count among male and female children were 1583 (1021-2188) and 1963 (1053-2124) cells/mm3 respectively. Amongst the HIV infected children 42 (20 male and 22 female) out of the 50 children were under antiretroviral therapy during this study, and 8 (16%) were ART naïve. As controls44 HIV negative children consisting of 21 females with a median age of 10 months (IQR, 6-23) and 23 males with a median age of 10 months (IQR 6-19) were also recruited. All participants completed the HBV vaccination schedule were both HBsAg and HBV envelope antigen (HBeAg) negative.

Comparative analysis of the concentration of anti-HBs and the HBV vaccine response rate between HIV positive and negative children

Four weeks following the last HBV vaccine dosethe protectiveanti-HBs seroconversion (anti-HBs ≥10 mUI/ml) rate was 36 % in the HIV+ compared with 96 % in their negative counterparts (Figure 1A). When anti-HBs concentrations were categorized as i) no response, ii) < 10 mIU/ml and iii) > m10 IU/ml; significantly higher frequencies of HIV infected children were either non-responders or had non-protective levels of ant-HBs. With respect to IgM antibody responses HIV+ positive children showed HBsAg specific IgM antibody responses (P=0.99) comparable to seronegative children. On the other hand, HBsAg specific IgG responses were significantly (P<0.0001) reduced in HIV infected children relative to the health controls (Figure 1B).

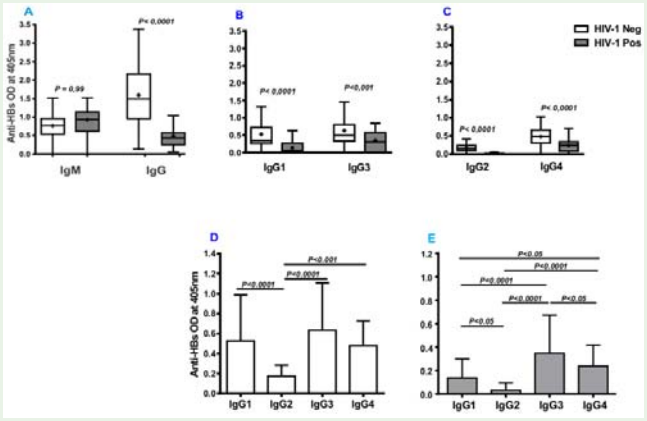

Figure 1: Hepatitis B surface antigen specific antibody responses in HIV negative and positive children after childhood vaccination against HBV infection.
In (A) proportion of anti-HBs responders in the study population. In (B) HBsAg specific IgM and IgG isotype responses. In (C) a correlation between CD4+/CD8+ ratio and HBsAg specific IgM antibody response whilst (D) is a correlation between CD4+/CD8+ ratio and anti-HBs IgG response. p-value at 0.05 significant level.

HBs Ag specific IgG subclass antibody responses in HIV infected children

HBsAg specific IgG subclass responses including IgG1 (P<0.0001), IgG2 (0.0001), IgG3 (P<0.001) and IgG4 (P<0.0001) responses specific to HBsAg were significantly higher in HIV negative children than their positive counter parts (Figure 1B and 1C). The pattern of hepatitis B surface antigen specific IgG antibody subclass responses in HIV-1 negative children was IgG3=IgG1=IgG4>IgG2 compared to the HIV positive children which was IgG3>IgG4=IgG1>IgG2.

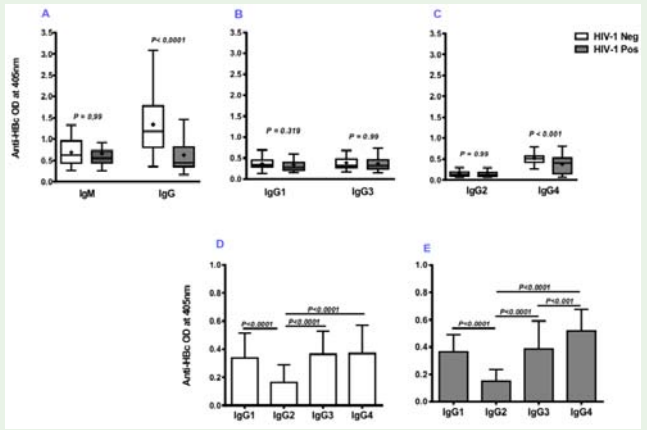

Figure 2: Hepatitis B surface antigen specific IgG subclass responses in HIV negative and positive children after childhood vaccination against HBV infection.
In (A) comparison between HIV negative and positive children on their HBsAg specific IgG1 and IgG3 subclass responses. (B) is a similar comparison but this time around for HBsAg specific IgG2 and IgG4 subclass responses. In (C) the profile of hepatitis B surface antigen specific IgG subclass responses is shown for HIV negative children whilst (D) shows similar data for the positive children. P: p-value at 0.05 significant levels.

Hepatitis B surface antigen specific IgM and IgG antibody responses amongst HIV-1 infected ARV therapy naive and treated children

Antiretroviral treated HIV-1 infected children showed similar HBsAg specific IgM and IgG antibody response like Antiretroviral Therapy (ART) naïve participants (P=0.99) (Figure 3A). However when HBsAg specific IgG subclass responses were considered apart from the IgG3 subclass responses in ART treated children which was significantly higher (P=0.0035) than ART naïve children, all other HBsAg specific IgG subclass responses including IgG2 (P=0.99), IgG1 (P=0.137) and Ig4 (p=0.449) were similar between the two groups (Figure 3B). Thus the overall effect of ART was a significant improvement in HBsAg specific IgG3 subclass responses.

HBsAg specific antibody response profiles according to age of the vaccinated children

We next categorize the participants into three main age groups consisting of 4 to 12, 13 to 24 and 25 to 60 months old respectively. The overall HBsAg specific IgM antibodies responses were similar between the different age groups in both HIV infected and negative (Figure. 4A). In contrast for all three age groups IgG antibody responses were significantly higher in the HIV negative than HIV positive children. The same trend was also observed with IgG1, IgG2 and IgG4 subclass responses (Figure 4B, C, D & F). On the other hand no difference is observed between the HIV positive and negative children with respect to HBsAg specific IgG3 subclass responses (Figure 4E).

Hepatitis B core antigen (HBc) specific IgM and IgG antibody responses in HIV infected children

Less than 10% of both HIV positive and negative children showed antibodies specific to the core antigen. Where HIV infected children showed similar HBcAg specific IgM responses (P=0.99) like the negative children (Figure 5A), HBcAg specific IgG antibody responses were significantly higher in the negative children (P=0.0001). The majority of the children had as consequence also extremely low prevalence of HBcAg specific IgG subclasses responses (compare Figure 5 B, C, D& E). HBc A specific IgG subclass responses were similarly between HIV negative and positive children thereby suggestion low infection with HBV (Table 1).

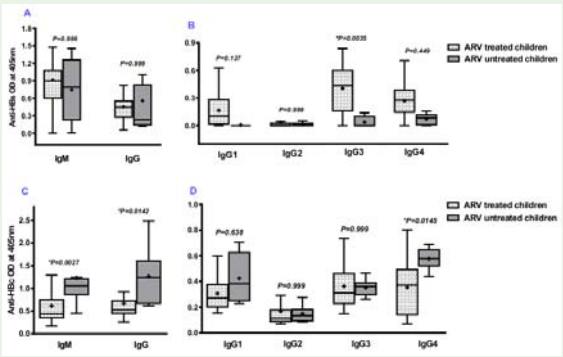

Figure 3: Effect of ART on HBsAg specific antibody response in HIV infected children. In (A) data for HBsAg specific IgM and IgG antibody responses are shown whilst (B) represents the profile of HBsAg specific IgG subclass responses in both ART treated and therapy naïve HIV infected children. P: p-value at 0.05 significant level.

Citation: Tchouangueu TF, Lissom A, Ngu LN, Priso GDN, Tchadji JC, Ngane CSS, et al. Vertically Acquired HIV Infection in Children Modulates Hepatitis B Surface Antigen Specific IgG Subclass Distribution After Early Childhood Vaccination Against Hepatitis B Virus Infection. Ann Clin Exp Immunol. 2018; 2(1): 1002.

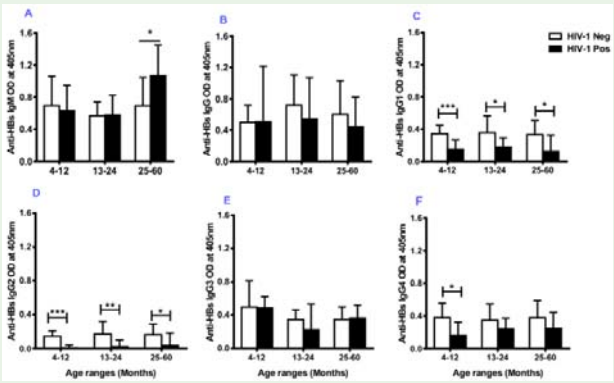

Figure 4: Hepatitis B surface antigen specific antibody responses in both HIV negative and positive children according to age.
In (A) IgM antibody, (B) IgG antibody, (C) IgG1 antibody, (D) IgG2 antibody, (E) IgG3 antibody and (F) IgG4 antibody responses. P: p-value at 0.05 significant level.

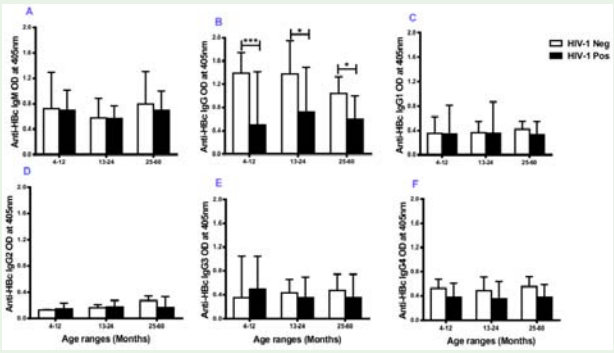

Figure 5: Proportion ofHBcAg specific antibody subclass response rates in HIV negative and HIV infected children.
In (A) comparative analysis of HBcAg specific IgM and IgG responses is shown for both HIV positive and negative children. In (B) proportion based on HBcAg specific IgG1 subclass response; (C) proportion based on HBcAg specific IgG2 subclass; (D) proportion based on HBcAg specific IgG3 subclass and (E) proportion based on HBcAg specific IgG4 subclass responses.

Discussion

In this study we have assessed the rate, concentration and IgG antibody subclass responses specific to HBV vaccine in 50 HIV infected as well as 44 HIV negative children one month after the last pediatric HBV vaccine dose. A direct consequence of HIV infection was a significant reduction in the rate, concentration and the profile of IgG subclass responses. The sero-protective HBV vaccine response rate (anti-HBs ≥10 UI/ml) in the HIV infected children was 36% compared to 92 % in healthy controls. The protective response rate to HBV vaccine as reported in terms of a seroconversion with anti-HBs antibodies > 10UI/mL is usually in the range of 90 to 95%39. However in HIV infected individuals the induction of sero-protective levels of anti-HBs antibodies as a consequence of HIV driven immune-depletion is severely impaired. The greater majority of the children (>60 % for HIV positive versus <10% for negative children) had serum anti-HBs IgG levels less than 10 IU/l. In this study the induction anti-HBs antibody following Child Hood vaccination against HBV as previously reported30-34 was severely impaired in children vertically infected with HIV.

Given the above HBV vaccine response rate and low levels of HBsAg specific IgG antibodies the need arises to regularly monitor anti-HBs following the last HBV vaccine dose in HIV infected children to identify non-immunized individuals for revaccination. There is also a need for sustained monitoring of HIV infected children to long understand the impact of poor vaccine responses on the emergence of HBV related diseases and cancers. In addition despite comparatively similar levels of IgM specific to HBsAg in both HIV positive and negative children levels of IgG antibodies were also significantly reduced (P=0.0001) in the HIV infected children (Fig 1B). This was mirrored in the profile of HBsAg specific IgG subclass responses including IgG1 (P=0.0001), IgG2 (p=0.0001), IgG3 (P=0.0001) and IgG4 (P=0.0001) which were all significantly lower in the HIV infected children [33] (Figure 2 A&B).

Since IgM responses were similar between the two groups the differences observed in HBsAg specific IgG and IgG subclass responses is probably as a result of an impaired ability of HIV infected children to generate adequate IgG antibody responses to HBV vaccine [34]. This is probably because the IgG subclass content is dependent upon the functions of the germinal center which is responsible for the functional diversification of IgG antibody responses and the generation of class switched long lasting memory B cells [35].

Table 1: Study population characteristics.

Variable	HIV- (n=44)		HIV+ (n=50)	
Gender	Male	Female	Male	Female
Participants n (%)	23 (52.27)	21 (47.73)	24 (48)	26 (52)
Median age (IQR)in months(a)	10(6-19)	10 (6-23)	48(34-59) ****	30 (20-49) **
HIV status	Negative	Negative	Positive	Positive
CD4+(IQR), in Cells/mm3	N/A	N/A	1583 (1021 -2188)	1963 (1053-2124)
ART n (%)	N/A	N/A	20 (83.33)	22 (84.61)

n: Number; IQR: Inter Quartile Range; ART: Antiretroviral Therapy; **:P<0.001; ****:P<0.0001; (a): Mann-Whitney test comparing median age of a given gender between groups.

The induction of IgG subclass responses which is a critical step in memory B cell generation also occurs in the light zone of the germinal center where their B cells (centrocytes) would undergo class switch recombination [36]. In this zone interactions between follicular helper CD4+ T cells, follicular dendritic cells and primed B cells determine the IgG subclass content and the durability of memory B cells35. HIV have been reported to infect cells of the germinal center such as follicular helper T cells leading to severe immune dysfunction. In this light impaired vaccine mediated humoral immune responses in HIV infected people have been linked to the loss of memory B cells during disease progression [37-39]. This probably suggests that HBV vaccine specific immune response development in HIV infected children might also be impaired with respect to IgG subclass generation (class switch). This implies that HBsAg specific IgM antibody responses might be the main antibody isotype responsible for HBV vaccine immunity in HIV infected individuals.

In both groups HBsAg specific IgG subclass antibodies were mainly restricted to IgG1, IgG3 and IgG4 subclasses. The IgG2 level of anti-HBs was the lowest IgG subclass for both groups (Figure 2 C&D). Whereas levels of HBsAg specific IgG1 and IgG3 were similar in the vaccinated HIV negative children these subclasses of anti-HBs in the HIV infected children was dominated by IgG3 with IgG1 being severely diminished. This suggests that in contrast to healthy controls IgG3could be the main anti-HBs IgG subclass following pediatric vaccination of HIV infected children against HBV.

The implementation of mass immunization programs, which have been recommended by the World Health Organization since 1991, have dramatically decreased the incidence of HBV infection among infants, children, and adolescents in many countries40. It is known that, immune responses to most vaccine are impaired in individuals infected with HIV [41,42]. Our data suggest that HBsAg vaccine specific-humoral immune response maturation might also be impaired in HIV-1 infected children. Successful vaccination using recombinant HBV vaccine is a T-cell-dependent process [43] and the reduction in HBV vaccine mediated antibody responses among HIV-infected children might be a consequence HIV driven deregulation of the functions of the germinal center [44] where helper CD4+ T cells are known to provide critical help during the maturation of antibody responses. Since there is a direct correlation between HBV directed humoral and cell mediated immunity20 such a dysfunction of the germinal center might lead to a decrease in HBsAg specific IgG1, IgG2, IgG3 and IgG4 due to a limitation in class-switching after HBV vaccination of HIV-1 infected children.

Moreover, ongoing HIV replication is associated with B cell deregulations, increased B cell activation and turnover, and an increase in immature and transitional B cells [45,46]. This results in the loss of memory B cells and the decrease in levels of previously induced antibody [37,38,45,47]. HIV-1 Nef has also been shown to directly perturb B-cell class switching [48-51]. In fact, Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells [52].

In this study IgG4 response was unusually elevated amongst the IgG subclass antibody responses in both HIV infected and negative children. This is probably due to the fact that IgG4 is associated with chronically exposed antigens and thus might be involved in immune response down-regulation or tolerance induction function [53].

Treatment of HIV-1 infected children with antiretroviral drugs therapy improved both anti-HBs and anti-HBc specific antibody responses. In antiretroviral therapy naive HIV-1 infected children there was a significant reduction in HBsAg specific IgG3 responses relative to their ART treated counterparts. Similarly HBcAg specific IgM, IgG and IgG4 were also significantly lower in ART therapy naive HIV-1 infected children relative to those ART treated. ART therapy is known to suppress HIV-1 replication and the reduction of pro-inflammatory cytokines in lymphoid tissue [54], leading to an improvement in immune responses [55]. Improved HBV vaccine specific antibody responses in ART treated children is an indication of partial immune restoration following treatment of HIV infected children.

Our study had some limitations, one of which being that only plasma was analyzed for HBV vaccine specific immune responses. This might not be sufficient to decipher all the problems related to poor HBV vaccine responses in HIV infected children. Secondly the relatively small sample size of the study population might not be a true representation of the entire population of HIV infected children in Cameroon. Never the less our study assessed the humoral response to HBV vaccine as well as possible exposure to HBV infection during early infancy.

Conclusion

We have shown that anti-HBs immune response rate, concentration and HBsAg specific IgG subclass antibody responses are impaired in HIV infected children. Thus the HIV infected children'sHBV vaccine immune responses might not be as effective as that of their negative counterpart for reasons that include lack of immunologic memory, immaturity and low level HBsAg specific IgG subclass antibody responses. Given that anti-HBs titer is a strong correlate for predicting protection after HBV vaccination; HIV infected children should be tested regularly for anti-HBs concentration in order to give booster injection when titers are below 10mIU/ml. There is also need for long-term monitoring to establish the absence of clinically significant breakthrough instances of HBV infections.

Acknowledgement and Disclosure

We thank all consented participants who participate in this study. We would like to thank the personnel of CIRCB and health facilities for their collaboration and help in collecting blood samples.

Funding

This work This project was funded by grants from CIRCB, EDCTP (grant #TA.2010.40200.016) TWAS(#12059RG/bio/af/acG) and Canada grand challenge (#0121-01); to Godwin W N chinda; from Korea-Africa cooperation grant (NRF-2013K1A3A1A09076155) to Chae Gyu Park; and then from the Center for Nano Biotechnology Research (CNBR) of ASU for grant # NSF-CREST (HRD-241701) and grant # NSF-AGEP (1432991 BKR) of National Science Foundation to Alain Bopda Waffo. This project was also largely funded by the Cameroonian government through CIRCB.

References

1. Smith CJ, Ryom L, Weber R. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. Lancet. 2014; 384: 241-248.

2. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev. 2006; 28: 112-125.

3. Amponsah-Dacosta E, Lebelo RL, Rakgole JN, Burnett RJ, Selabe SG, Mphahlele MJ. Evidence for a change in the epidemiology of hepatitis B virus infection after nearly two decades of universal hepatitis B vaccination in South Africa. Journal of medical virology. 2014; 86: 918-924.

4. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012; 30: 2212-2219.

5. McGovern BH. The epidemiology, natural history and prevention of hepatitis B: implications of HIV coinfection. Antiviral therapy. 2007; 12: H3-13.

6. Puoti M, Airoldi M, Bruno R. Hepatitis B virus co-infection in human immunodeficiency virus-infected subjects. AIDS Rev. 2002; 4: 27-35.

7. Herrero-Martinez E, Sabin CA, Lee CA, Jones IM, Pillay D, Emery VC. The effect of highly active antiretroviral therapy for HIV on the anti-HCV specific humoral immune response. Journal of medical virology. 2004; 72: 187-193.

8. Burnett RJ, Francois G, Kew MC, et al. Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation. Liver international : official journal of the International Association for the Study of the Liver. 2005; 25: 201-213.

9. Diamant EP, Schechter C, Hodes DS, Peters VB. Immunogenicity of hepatitis B vaccine in human immunodeficiency virus-infected children. The Pediatric infectious disease journal. 1993; 12: 877-878.

10. Arrazola MP, de Juanes JR, Ramos JT, Aragon AJ, Garcia de Codes A. Hepatitis B vaccination in infants of mothers infected with human immunodeficiency virus. Journal of medical virology. 1995; 45: 339-341.

11. Thimme R, Spangenberg HC, Blum HE. Hepatitis B or hepatitis C and human immunodeficiency virus infection. Journal of hepatology. 2005; 42: S37-44.

12. Scolfaro C, Fiammengo P, Balbo L, Madon E, Tovo PA. Hepatitis B vaccination in HIV-1-infected children: double efficacy doubling the paediatric dose. Aids. 1996; 10: 1169-1170.

13. Abzug MJ, Warsaw M, Rosenblatt HM. Immunogenicity and immunologic memory after hepatitis B virus booster vaccination in HIV-infected children receiving highly active antiretroviral therapy. The Journal of infectious diseases. 2009; 200: 935-946.

14. Giacomet V, Masetti M, Nannini P. Humoral and cell-mediated immune responses after a booster dose of HBV vaccine in HIV-infected children, adolescents and young adults. PloS one. 2018; 13: e0192638.

15. Morell A, Roth-Wicky B, Skvaril F. Immunoglobulin G subclass restriction of antibodies against hepatitis B surface antigen. Infection and immunity. 1983; 39: 565-568.

16. Guidotti LG, Isogawa M, Chisari FV. Host-virus interactions in hepatitis B virus infection. Current opinion in immunology. 2015; 36: 61-66.

17. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunology today. 1996; 17: 138-146.

18. Urban M, Winkler T, Landini MP, Britt W, Mach M. Epitope-specific distribution of IgG subclasses against antigenic domains on glycoproteins of human cytomegalovirus. The Journal of infectious diseases. 1994; 169: 83-90.

19. Jefferis R, Pound J, Lund J, Goodall M. Effector mechanisms activated by human IgG subclass antibodies: clinical and molecular aspects. Review article. Ann Biol Clin (Paris). 1994; 52: 57-65.

20. Gelinas L, Abu-Raya B, Ruck C, Cai B, Kollmann TR. Hepatitis B Virus Vaccine-Induced Cell-Mediated Immunity Correlates with Humoral Immune Response following Primary Vaccination during Infancy. ImmunoHorizons. 2017; 1: 45-52.

21. Young HA, Hardy KJ. Role of interferon-gamma in immune cell regulation. Journal of leukocyte biology. 1995; 58: 373-381.

22. Collins AM. IgG subclass co-expression brings harmony to the quartet model of murine IgG function. Immunology and cell biology. 2016; 94: 949-954.

23. Berkowska MA, Driessen GJ, Bikos V.Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. Blood. 2011; 118: 2150-2158.

24. Njom Nlend AE, Nguwoh PS, Ngounouh CT. HIV-Infected or -Exposed Children Exhibit Lower Immunogenicity to Hepatitis B Vaccine in Yaounde, Cameroon: An Appeal for Revised Policies in Tropical Settings? PloS one. 2016; 11: e0161714.

25. Kerneis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boelle PY. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2014; 58: 1130-1139.

26. Pollack TM, Trang le TT, Ngo L, Cuong do D, Thuy PT, Colby DJ. Response to hepatitis B vaccination among HIV-infected adults in Vietnam. J Virus Erad. 2016; 2102-106.

27. Whittle HC, Inskip H, Hall AJ, Mendy M, Downes R, Hoare S. Vaccination against hepatitis B and protection against chronic viral carriage in The Gambia. Lancet. 1991; 337: 747-750.

28. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? The Journal of infectious diseases. 1999; 179: 489-492.

29. Tsai TH, Huang CF, Wei JC. Study of IgG subclass profiles of anti-HBs in populations with different HBV infection status. Viral immunology. 2006; 19: 277-284.

30. Rey D, Krantz V, Partisani M. Increasing the number of hepatitis B vaccine injections augments anti-HBs response rate in HIV-infected patients. Effects on HIV-1 viral load. Vaccine. 2000; 18: 1161-1165.

31. Wilson CM, Ellenberg JH, Sawyer MK. 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. 2001; 29:123-129.

32. Arundel C, Lam PH, Khosla R. Association of 30-Day All-Cause Readmission with Long-Term Outcomes in Hospitalized Older Medicare Beneficiaries with Heart Failure. The American journal of medicine. 2016; 129: 1178-1184.

33. Bekele Y, Yibelta D, Bobosha Kl. T follicular helper cells and antibody response to Hepatitis B virus vaccine in HIV-1 infected children receiving ART. Sci Rep. 2017; 7: 1-11.

34. van den Berg R, van Hoogstraten I, van Agtmael M. Non-responsiveness to hepatitis B vaccination in HIV seropositive patients; possible causes and solutions. AIDS Rev. 2009; 11: 157-164.

35. French MA, Tjiam MC, Abudulai LN, Fernandez S. Antiviral Functions of Human Immunodeficiency Virus Type 1 (HIV-1)-Specific IgG Antibodies: Effects of Antiretroviral Therapy and Implications for Therapeutic HIV-1 Vaccine Design. Frontiers in immunology. 2017; 8: 780.

36. Hamel KM, Liarski VM, Clark MR. Germinal center B-cells. Autoimmunity. 2012; 45: 333-347.

37. De Milito A, Nilsson A, Titanji K. Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. Blood. 2004; 103: 2180-2186.

38. Titanji K, De Milito A, Cagigi A. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood. 2006; 108: 1580-1587.

39. Hart M, Steel A, Clark SA. Loss of discrete memory B cell subsets is associated with impaired immunization responses in HIV-1 infection and may be a risk factor for invasive pneumococcal disease. Journal of immunology. 2007; 178: 8212-8220.

40. Hepatitis B. 2015. http://www.who.int/mediacentre/factsheets/fs204_Jul2014/en/.

41. Geretti AM, Doyle T. Immunization for HIV-positive individuals. Curr Opin Infect Dis. 2010; 23: 32-38.

42. Abzug MJ. Vaccination in the immune compromised child: a probe of immune reconstitution. The Pediatric infectious disease journal. 2009; 28: 233-236.

43. Tayal SC, Sankar KN. Impaired response to recombinant hepatitis B vaccine in asymptomatic HIV-infected individuals. Aids. 1994; 8: 558-559.

44. Lao-araya M, Puthanakit T, Aurpibul L, Sirisanthana T, Sirisanthana V. Antibody response to hepatitis B re-vaccination in HIV-infected children with immune recovery on highly active antiretroviral therapy. Vaccine. 2007; 25: 5324-5329.

45. Cagigi A, Nilsson A, De Milito A, Chiodi F. B cell immunopathology during HIV-1 infection: lessons to learn for HIV-1 vaccine design. Vaccine. 2008; 26: 3016-3025.

46. Malaspina A, Moir S, Ho J. Appearance of immature/transitional B cells in HIV-infected individuals with advanced disease: correlation with increased IL-7. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103: 2262-2267.

47. Moir S, Malaspina A, Pickeral OK. Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF super family. The Journal of experimental medicine. 2004; 200: 587-599.

48. Shen X, Tomaras GD. Alterations of the B-cell response by HIV-1 replication. Curr HIV/AIDS Rep. 2011; 8: 23-30.

49. Xu W, Santini PA, Sullivan JS. HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells via long-range intercellular conduits. Nature immunology. 2009; 10: 1008-1017.

50. Moir S, Fauci AS. Nef, macrophages and B cells: a highway for evasion. Immunology and cell biology. 2010; 88: 1-2.

51. Qiao X, He B, Chiu A, Knowles DM, Chadburn A, Cerutti A. Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. Nature immunology. 2006; 7: 302-310.

52. Moir S, Fauci AS. B cells in HIV infection and disease. Nature reviews Immunology. 2009; 9: 235-245.

53. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2009; 39: 469-477.

54. Andersson J, Fehniger TE, Patterson BK. Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. Aids. 1998; 12: F123-129.

55. Pontesilli O, Kerkhof-Garde S, Notermans DW. Functional T cell reconstitution and human immunodeficiency virus-1-specific cell-mediated immunity during highly active antiretroviral therapy. The Journal of infectious diseases. 1999; 180: 76-86.