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Homing defects of B cells in HIV-1 infected children impair vaccination responses

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ABSTRACT

Background: Successful vaccinations rely on antibody responses. Chemokine receptors play an important role in B cell homing to differentiation niches. We assessed CXCR4, CXCR5 and CCR6 expression on B cells during HIV-1 infection and relate it to antibody responses against a HBV vaccine.
Methods: Blood was obtained from 54 healthy controls and 38 ART-treated HIV-1 infected children, aviremic (n = 25) or viremic (n = 13). Frequency of naïve and memory B cell subsets was studied by immunostaining. Homing capacity of blood B cells to lymphoid and inflamed tissues was evaluated through CXCR4, CXCR5 and CCR6 expression. Plasma CXCL12 and CXCL13 levels and antibody titers to HBV antigen were determined by ELISA.
Results: The frequency of naïve and resting memory (RM) B cells in ART treated children was comparable to control subjects. Profound defects in the homing phenotypes of naïve and memory B cells were identified, with lower CXCR4 and CXCR5 expression. Increased CXCL13 levels were observed in infected children, inversely correlating to CXCR5 expressing B cell subpopulations. Antibody titers to HBV vaccine correlated with frequency of resting and switched memory B cells in HIV-1 infected children.
Conclusions: Homing defects of B cells to germinal center may underlie impaired vaccine responses during HIV-1 infection.

1. Introduction

Pathological alterations of B cell subpopulations, which may be at the root for impaired responses to childhood vaccines during HIV-1 infection in children, include depletion of resting memory B cells [1] and expansion of immature transitional B cells [2]. Elevated frequencies of activated and tissue-like memory B cells are also frequent in HIV-1 infected individuals. Tissue-like memory B cells respond poorly to B cell stimuli and express high levels of receptors inhibiting B cell responses [3].

Migration of B cells within the germinal center (GC), to lymphoid tissues or bone marrow (BM) is governed by chemokine receptors and their respective ligands [4–5]. CXCR4 plays a critical role in B cell development in BM; lack of CXCR4 expression in mice leads to increased release of premature B cells from the BM into the circulation [6]. CXCR4 is also important for trafficking of plasma cells from secondary lymphoid organs to the BM, as shown by accumulation of plasma cells in spleen and blood of CXCR4 deficient mice [7]. CXCR5 is relevant for B cell activation through B cell receptor (BCR) signaling [8] and migration to the GC light zone [9] where B cells access antigen at the surface of follicular dendritic cells (FDCs) [10]. Interaction of CCR6 expressing B cells with the chemokine ligand CCL20 directs B cells to inflamed tissues [11].

Very few studies have addressed whether defects in migration of B cells to organs important for B cell maturation and differentiation impact on the capacity of HIV-1 infected individuals to respond to vaccination and maintain physiological levels of serological memory to vaccines. A study conducted in adult HIV-1 infected patients showed that the homing capacity of B cells was impaired [12]. In particular, a decreased CXCR5 expression was observed in peripheral B cells; CXCR5 down-regulation occurred

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in parallel with increased expression of its ligand, CXCL13 [12]. These results suggested that CXCR5 down-regulation in B cells was mediated by an increased expression of its ligand.

Whether ART provided to HIV-1 infected children leads to improved B cell responses to childhood vaccines is yet not fully clarified [1,13]. In the present study, we assessed the expression of chemokine receptors important for B cell maturation and function, CXCR4, CXCR5 and CCR6 in cells from HIV-1 infected children receiving ART or naïve to treatment. The results showed a significantly reduced expression of CXCR4 and CXCR5 on B cells from HIV-1 infected children receiving ART which correlated with impaired response to hepatitis B virus (HBV) vaccination.

2. Methods

2.1. Study participants

A cross-sectional study was conducted in 54 healthy controls and 38 ART treated HIV-1 infected children. Specimens from all children were collected six months after completion of a HBV vaccination program in Ethiopia, according to previously published details [13]. All children received 3 doses (each of 10 mcg) of Hepatitis B vaccine (rDNA), (Serum Institute of India, Pune 411 028, India) which consists of purified surface HBV antigen with 4 weeks' interval (accelerated vaccination schedule) in between the doses. There was no severe adverse event reported upon vaccination. The children received treatment with ART as indicated in Table 1; the drugs were manufactured in India by HETERO Drugs Limited, Hyderabad, India. The study was conducted at the Zewditu Memorial Hospital and the Pediatric department of All Africa Leprosy, Tuberculosis and Rehabilitation Training Center (ALERT) in Addis Ababa, Ethiopia. Age matched healthy controls were recruited from two child care centers in Addis Ababa. Immunological and virological characteristics of participants are shown in Table 1. The viral load was undetectable in 25 of the HIV-1 infected children whereas the additional 13 children presented with a variable degree of viremia.

A blood sample was collected from all children. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll gradient centrifugation (Lymphoprep, Axis-Shield Poc AS, Oslo, Norway) and stored in liquid nitrogen at −196 °C for later use. Plasma specimens were stored at −80 °C.

HIV-1 RNA copies in blood were measured using an automated m2000sp Abbott Real-Time HIV-1 assay system following the manufacturer's protocol (Abbot Laboratories, Abbot Park, IL). The lower detection limit of this assay was 40 copies/ml.

There were no significant baseline differences between the two groups of viremic and aviremic patients in relation to age, CD4 counts, months on pre-ART and months on ART (Table 1).

The project proposal was reviewed and approved by the ethical review committees of the Armauer Hansen Research Institute (AHRI) Addis Ababa, Addis Ababa Health Bureau Ethics Board and National Research Ethics Review Committee of Ethiopia. Written informed consent was obtained from parents or guardians of study participants following an explanation of study purpose, benefit and possible discomfort. The ethical committee at Karolinska Institutet approved the laboratory studies of the collected specimens.

2.2. Immunophenotyping of B-cells and homing receptors

PBMCs were stained using the following monoclonal antibodies: anti-CD19 PE (H1B19), anti-CD10 FITC (W8E7), anti-CD21 PE-Cy7 (B-ly4), anti-CD27 BV421 (M-T271), anti-CXCR4 BV711 (12G5), anti-CXCR5 APC-R700 (RF8B2), anti-CCR6 PE-CF594 (11A9), anti-IgM APC (G20-127), all from BD, and LIVE/DEAD® Fixable Near Infrared dead Cell Stain (Molecular Probes by Life Technologies, OR, USA). Stained cells were washed with phosphate-buffered saline (PBS) before fixation in 2% paraformaldehyde. All antibodies were used at concentrations determined after titration experiments. Matched isotype controls were used to set the gating strategies. Fluorescence intensities were measured with LSRII (Becton Dickinson, San Jose, California) and data analyzed using FlowJo, Version 9.9.4 (Tree Star Inc, Ashland, Oregon). Gating strategies are presented in Supplementary Figs. 1 and 2.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2019.03.027.

Subpopulations of B cells were characterized as: Naïve B cells (CD19+CD10-CD21+CD27-); Resting memory B cells (CD19+CD10-CD21+CD27+, RM); Activated memory B cells (CD19+CD10-CD21-CD27+, AM); Tissue-like memory B cells (CD19

Table 1
Demographic and clinical characteristics of healthy controls and HIV-1 infected children.

Characteristic	Controls (N = 54)	HIV-1+ avir (N = 25)	HIV-1+vir (N = 13)
Age (years): Median (Range)	6.0 (4.0–8.0)	7.0 (4.0–9.0)	7.0 (5.0–8.0)
Gender			
Male	35 (64.8%)	15 (60%)	7 (54%)
Female	19 (35.2%)	10 (40%)	6 (46%)
CD4 Count (cells/ul): Median (Range)	ND	977 (361–2744)	798 (243–1752)
WHO stage			
I	NA	14 (56%)	9 (69%)
II		9 (36%)	3 (23%)
III		2 (8%)	1 (8%)
ART regimen			
AZT+3TC+NVP	NA	19 (76%)	12 (92%)
AZT+3TC+EFV		3 (12%)	1 (8%)
ABC+3TC+EFV		2 (8%)	–
D4T+3TC+NVP		1 (4%)	–
Months on ART: Median (Range)	NA	53 (7–90)	55 (6–77)
Months on pre-ART: Median (Range)	NA	35 (0.5–100)	26 (7–69)
Viral load (RNA copies/ml): Median (Range)	NA	Undetectable	1770 (150–53500)

ND = not done; NA = not applicable; undetectable =<40 RNA copies/ml.
AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; ABC = Abacavir; D4T = Stavudine.

+CD10-CD21-CD27-, TLM) and switched memory B cells (CD19+CD10-CD27+IgM-).

2.3. Determination of plasma anti-HBs antibodies, CXCL12 and CXCL13 titers by ELISA

The Monolisa Anti-HBs Plus assay (Bio-Rad, France) was used to measure plasma levels of antibodies to the HBV surface antigen (HBs) which is included in the HBV vaccine. The assay was run according to the manufacturer's instruction. Samples scoring above 10 IU/L (1 log IU/L) of anti-HBs antibodies were considered positive.

The Human CXCL13 and CXCL12 DuoSet ELISA kits (R&D systems, Minneapolis, MN) were used to measure the plasma levels of CXCL13 and CXCL12 according to the manufacturers' instructions; all samples were analyzed in duplicate. The OD values were converted to concentrations using the Microplate manager version 6 (Bio-Rad, California, USA).

2.4. Statistical analyses

Demographic and clinical data were analyzed using SPSS version 25 (IBM Corp, Armonk, NY, USA). Normal distribution of flow cytometry and ELISA data was assessed using Kolmogorov-Smirnov test. Unpaired T-tests and ANOVA were used to assess the difference between groups. The Spearman correlation was applied to determine the relation of variables using GraphPad Prism version 7 (La Jolla, CA, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Abnormal frequencies of B cell subpopulations in HIV-1 infected children receiving ART

The frequency of total (CD19+) B cells was significantly lower in HIV-1 infected children compared to healthy controls with median values of 8.8% and 11.3%, respectively ($p < 0.001$) (Fig. 1). The frequencies of naïve and RM B cells were significantly reduced in HIV-1 infected children compared to healthy controls (64.7% vs 71.5%, $p = 0.04$; 21.1% vs 25.1%, $p = 0.03$, respectively) (Fig. 1). The frequency of exhausted B cell subsets was significantly higher in HIV-1 infected than healthy controls (AM 3.4% vs 1.6%, $p < 0.0001$; TLM 6.8% vs 1.8%, $p < 0.0001$).

The median frequencies of naïve and RM B cells were reduced in viremic (naïve 52.8% vs 71.5%, $p < 0.01$; RM 15.8% vs 25.1%, $p < 0.01$) but not in aviremic HIV-1 infected children compared with healthy controls. On the contrary, the frequencies of AM and TLM B cells in healthy controls were significantly lower compared to viremic (AM 1.6% vs 8.7%, $p < 0.0001$; TLM 1.8% vs 18.8%, $p < 0.0001$) HIV-1 infected children; the aviremic group had a higher frequency of TLM compared to healthy controls (TLM 3.3% vs 1.8%). Similar frequencies of naïve, RM, AM and switched memory B cells were observed between healthy con-

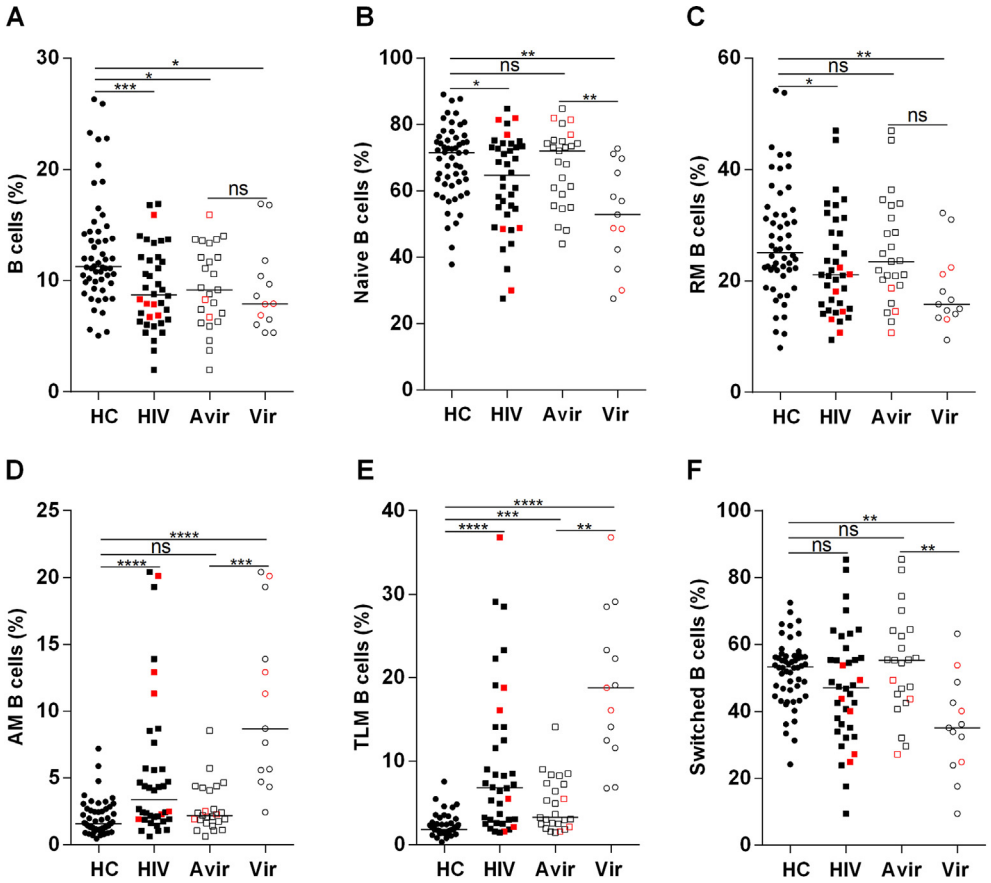

Fig. 1. B cell subpopulations in healthy controls and HIV-1 infected children. Frequency of B cell subpopulations in healthy controls (n = 54) and HIV-1 infected children (n = 38), aviremic (n = 25) or viremic (n = 13). The panels show: Total B cells (CD19+); naïve B cells (CD19+CD10-CD21+CD27-); Resting memory B cells (CD19+CD10-CD21+CD27+, RM); Activated memory B cells (CD19+CD10-CD21-CD27+, AM); Tissue-like memory B cells (CD19+CD10-CD21-CD27-, TLM) and switched memory B cells (CD19+CD10-CD27+IgM-). The red symbols show the frequencies of B cell subpopulations of HBV vaccine non-responders. The horizontal lines show the median values within the respective groups. ns = not statistical significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Table 2
Correlation of B cell subset frequencies with clinical parameters.

	Age (years)	CD4+T cell counts	HIV-1 RNA copies/ml	Time on ART
Total B cells	n.s.	n.s.	n.s.	n.s.
Naive	n.s.	n.s.	r = −0.58 p = 0.0002	r = −0.52 p = 0.0008
RM	n.s.	n.s.	r = −0.34 p = 0.04	r = 0.49 p = 0.002
AM	n.s.	n.s.	r = 0.79 p = 0.0001	n.s.
TLM	n.s.	n.s.	r = 0.78 p = 0.0001	n.s.
Switched memory	n.s.	n.s.	r = −0.51 p = 0.001	n.s.

n.s. = not significant.
RM = resting memory B cells; AM = activated memory B cells; TLM = tissue-like memory B cells.

trols and aviremic children. The frequencies of total CD19+ and RM B cells did not differ between viremic and aviremic HIV-1 infected children. However, viremic children had a significantly lower frequency of naïve and switched memory B cells compared to aviremic HIV-1 infected children ($p < 0.01$). Exhausted B cells, AM ($p < 0.001$) and TLM B cells ($p < 0.01$), were significantly expanded in viremic children compared to aviremic children.

We correlated the frequencies of B cell subsets with the clinical and immunological parameters presented in Table 1. The results of these correlations are shown in Table 2. A significant negative correlation was found between HIV-1 RNA copies/ml and frequencies of naïve ($p = 0.0002$), RM ($p = 0.04$) and switched memory ($p = 0.001$) whereas viremia was directly correlated with the expanded frequencies of exhausted AM ($p = 0.0001$) and TLM ($p = 0.0001$) B cells. A significant negative correlation was found between the time on ART and the frequency of naïve B cells ($p = 0.0008$) whereas the frequency of RM B cells directly correlated to the time spent on ART ($p = 0.002$).

3.2. Impaired expression of chemokine receptors important for B cell migration in HIV-1 infected children

We measured the potential capacity of B cells to migrate to BM, within the GC and to inflamed tissue using the expression of chemokine receptors CXCR4, CXCR5 and CCR6.

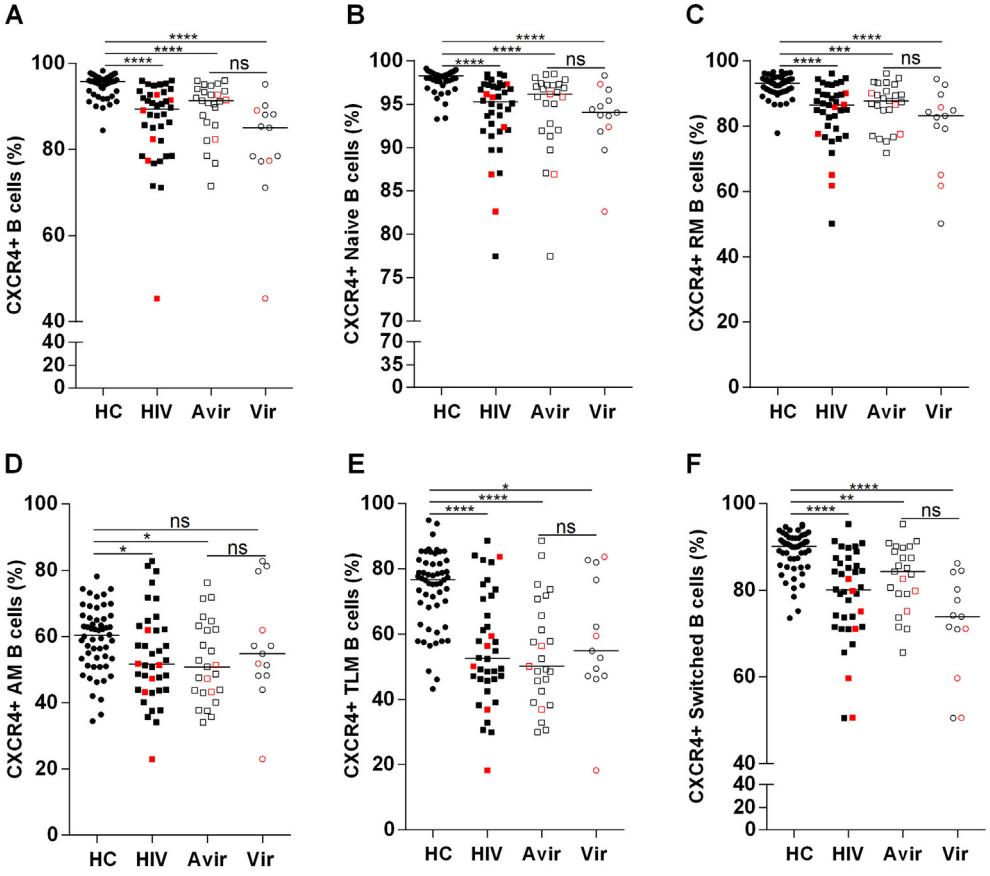

Fig. 2. Frequency of CXCR4 expressing B cell subpopulations in healthy controls and HIV-1 infected children. The expression of CXCR4 chemokine receptor was determined in B cell subpopulations of healthy controls (n = 54) and HIV-1 infected children (n = 38), aviremic (n = 25) and viremic (n = 13). The red symbols show the frequencies of CXCR4 + B cell subpopulations of HBV vaccine non-responders. The horizontal lines show the median values within the respective groups. ns = not statistical significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

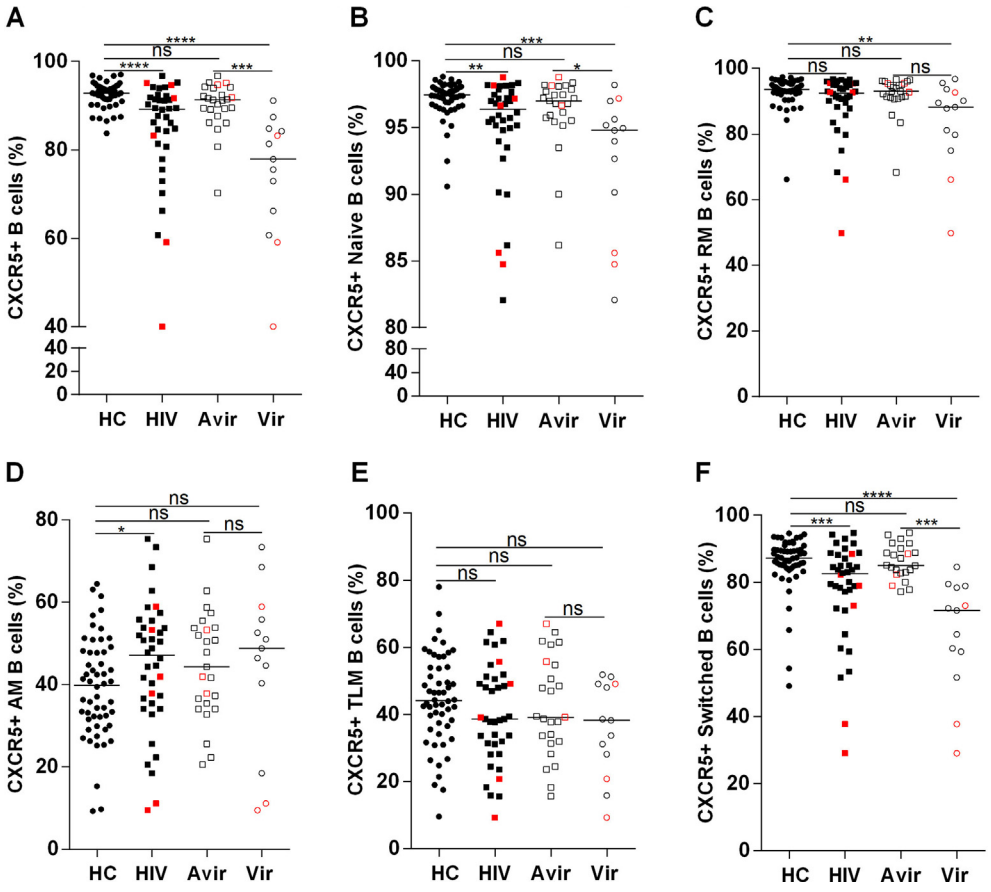

Fig. 3. Frequency of CXCR5 expressing B cell subpopulations in healthy controls and HIV-1 infected children. The expression of CXCR5 receptor was determined in B cell subpopulations of healthy controls (n = 54) and HIV-1 infected children (n = 38), aviremic (n = 25) and viremic (n = 13). The red symbols show the frequencies of CXCR5+ B cell subpopulations of HBV vaccine non-responders. The horizontal lines show the median values within the respective groups. ns = not statistical significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

CXCR4 expression on B cells (Fig. 2) was significantly reduced in HIV-1 infected children compared to healthy controls. In addition, there were no differences in CXCR4 expression of B cell subpopulations between aviremic and viremic HIV-1 infected children.

Interestingly, the frequencies of CXCR5+CD19+, naïve and switched memory B cells were significantly lower in HIV-1 infected children compared to controls (Fig. 3). On the contrary, the frequency of CXCR5+ AM B cells was significantly higher in HIV-1 infected children compared to controls. The frequencies of CXCR5 + RM (93.5% vs 92.5%) and TLM (44.1% vs 38.6%) B cells were however comparable in healthy controls and HIV-1 infected children. With the exception of AM and TLM, the frequency of CXCR5 expressing B cell subpopulations was significantly reduced in viremic children compared to healthy controls. Viremic children showed reduced frequencies of CD19+, naïve and switched memory B cells compared to aviremic HIV-1 infected children.

There was no difference in the frequencies of CCR6+ total, naïve, RM and switched memory B cells between healthy controls and HIV-1 infected children (Fig. 4). HIV-1 infected children, both aviremic and viremic, however, had a significantly higher frequency of CCR6+ AM (80.3% vs 62.8%, p < 0.0001) and CCR6+ TLM (91.9% vs 77.6%, p < 0.0001) B cells compared to healthy controls.

The simultaneous expression of CXCR4 and CXCR5 was evaluated on naïve and RM B cells (Fig. 5). Interestingly, the frequency of CXCR4+CXCR5+ naïve B cells was reduced in HIV-1 infected children, both aviremic (median 98.2%; range 83.5–99.4%; p < 0.01) and viremic (median 94.1%; range 84.6–99.2%; p < 0.0001), as compared to healthy controls (median 99.1%; range 92.1–99.7%). A difference was also observed for CXCR4+CXCR5+ RM B cells, with healthy controls (median 93.6%; range 66.8–98.6%) displaying a significantly higher frequency than aviremic (median 90.1.%; range 60.8–96.4%; p < 0.01) and viremic HIV-1 infected children (median 81.5%; range 51.2–96.8%%; p < 0.001).

The frequencies of B cell subpopulations expressing the different chemokine receptors were analyzed in relation to the length of treatment; only one significant correlation was identified for CXCR5+ B cells (p < 0.05).

3.3. CXCL12 and CXCL13 in plasma

Plasma levels of CXCL12 were higher in HIV-1 infected children compared to healthy controls with the median concentration of 2.45 log pg/ml (range 2.16–2.97 log pg/ml) and 2.36 log pg/ml (range 2.16–2.79 log pg/ml), respectively (Fig. 6A). However, CXCL12 concentration did not differ between aviremic and viremic children. The levels of CXCL13 were significantly higher (p < 0.001) in HIV-1 infected children (median 2.36 log pg/ml; range 2.01–3. 20 log pg/ml) compared to healthy controls (median 2.04 log pg/ml; range 1.65–2.56 log pg/ml). There was no difference in CXCL13 concentration between aviremic (median 2.44 log pg/ml) and viremic (median 2.47 log pg/ml) children (Fig. 6B).

We correlated clinical and laboratory parameters including age, CD4+ T cell counts, viral load and length of ART treatment (Table 1) with CXCL12 and CXCL13 levels; a positive correlation was only found between CXCL13 concentration and viral load (r = 0.65; p < 0.0001) (Fig. 6C). In contrast, the levels of CXCL13 and CXCL12 did not correlate with age, CD4+ T cell counts and length of ART treatment.

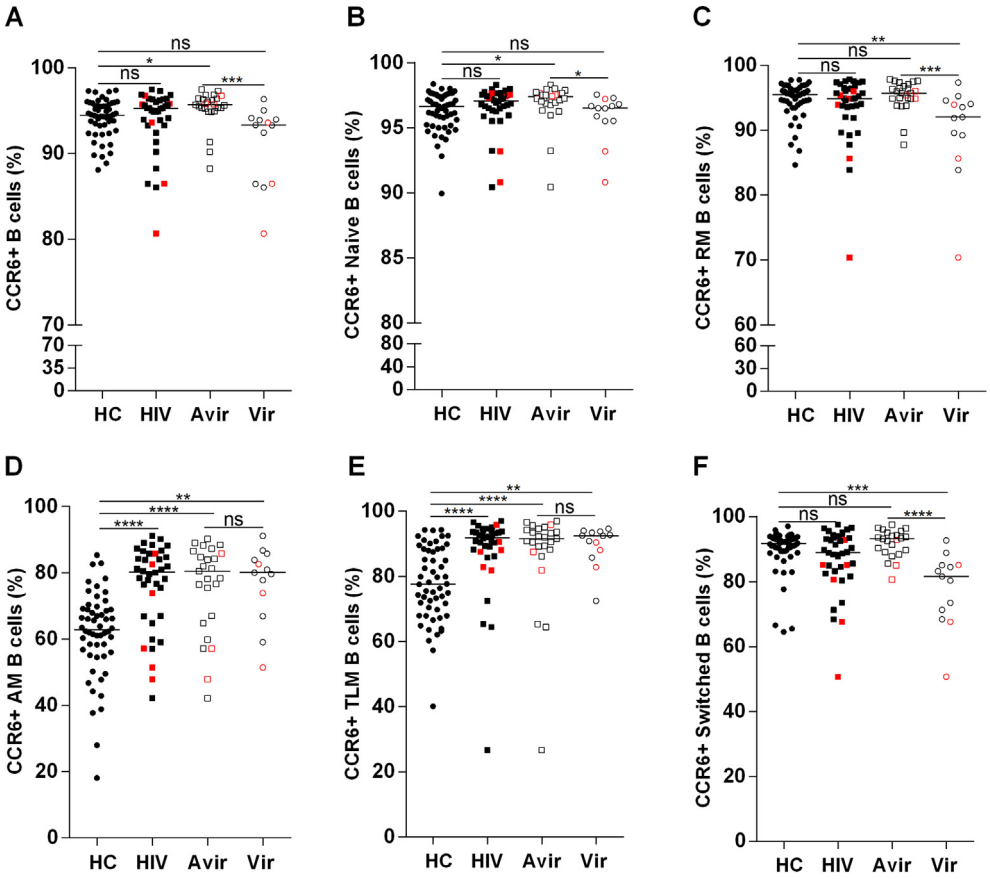

Fig. 4. Frequency of CCR6 expressing B cell subpopulations in healthy controls and HIV-1 infected children. The expression of CCR6 receptor was determined in B cell subpopulations of healthy controls (n = 54) and HIV-1 infected children (n = 38), aviremic (n = 25) and viremic (n = 13). The red symbols show the frequencies of CCR6+ B cell subpopulations of HBV vaccine non-responders. The horizontal lines show the median values within the respective groups. ns = not statistical significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

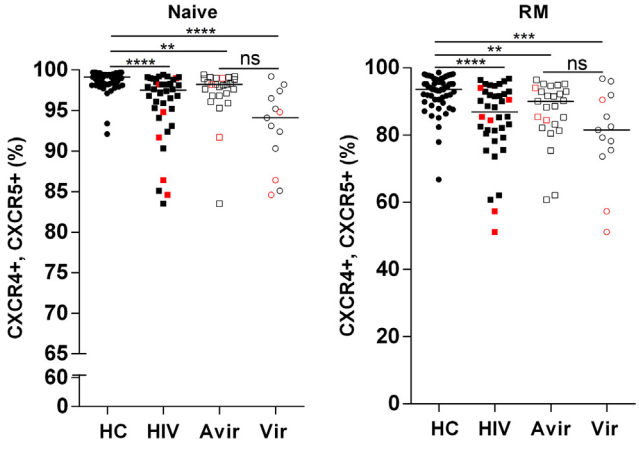

Fig. 5. CXCR4+CXCR5+naïve and resting memory cells in healthy controls and HIV-1 infected children. The frequencies of CXCR4+CXCR5+double expressing naïve and RM B cells were evaluated in healthy controls (n = 54) and HIV-1 infected children (n = 38), aviremic (n = 25) and viremic (n = 13). The red symbols show the frequencies of CXCR4+CXCR5+ B cell subpopulations of HBV vaccine non-responders. The horizontal lines show the median values within the respective groups. **p < 0.01, ***p < 0.001, ****p < 0.0001.

We assessed whether a correlation existed between CXCL13 levels and frequencies of B cell subpopulations and CXCR5+ B cell subpopulations. In HIV-1 infected children, but not in healthy controls, a positive correlation was found between CXCL13 levels and the frequencies of AM (p = 0.0004) and TLM (p = 0.0001) memory B cells (Table 3). In addition, CXCL13 levels were inversely correlated to naïve (p = 0.008) and switched memory (p = 0.005) B cells in HIV-1 infected children. Remarkably, in HIV-1 infected children all CXCR5+ B cell subpopulations showed a highly significant inverse correlation with CXCL13 levels (Table 3). In healthy controls only the CXCR5+ AM B cells showed a significant inverse correlation with CXCL13 levels (p = 0.03). We did not find any correlation between CXCL12 concentration and B cell subsets.

3.4. Correlation of plasma anti-HBs antibodies with B cell subsets and chemokine ligands

At six months from completed HBV vaccination, all 54 controls (median 2.99 log IU/L), 22 of 25 aviremic children (2.23 log IU/L) and 10 of 13 viremic children (1.93 log IU/L) presented with anti-HBs antibody levels above cut-off value. There was however a significant difference (p < 0.0001) in anti-HBs antibody levels between healthy controls and HIV-1 infected children (Fig. 6D).

The plasma CXCL13 levels were inversely correlated with anti-HBs antibody titers (r = −0.38; p = 0.004) in healthy controls. In HIV-1 infected children, CXCL12 levels correlated with titers of anti-HBs antibodies (r = 0.37; p = 0.03).

In HIV-1 infected children, the plasma concentration of anti-HBs antibodies significantly correlated with frequencies of RM B cells (p < 0.01) and switched memory B cells (p = 0.03). The frequency of CXCR4+CXCR5+ naïve B cells showed a correlation with anti-HBs titers in healthy controls (r = 0.32; p = 0.02) but not in HIV-1 infected children.

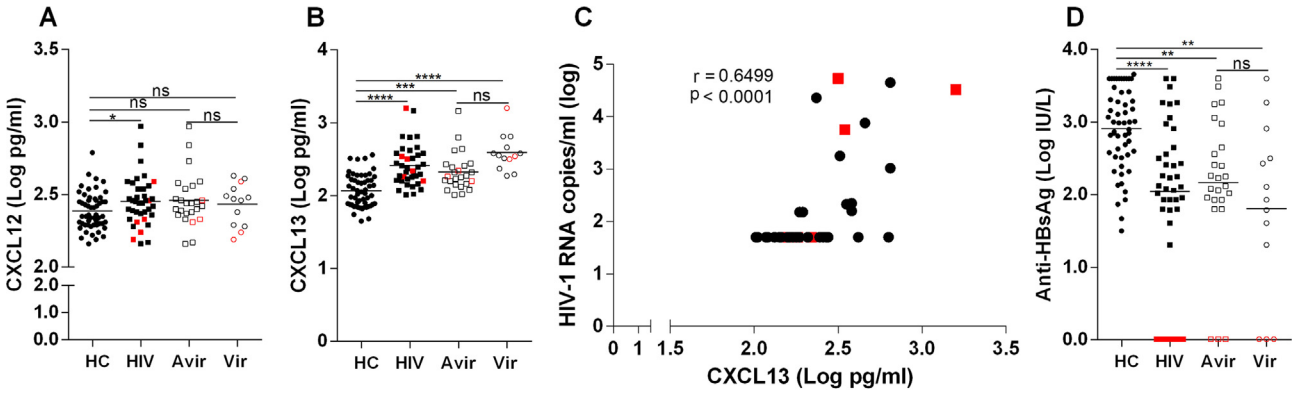

Fig. 6. CXCL13, CXCL12 and antibody levels to HBV vaccine in plasma of healthy controls and HIV-1 infected children. CXCL12 (A) and CXCL13 (B) were measured in plasma specimens from healthy controls (n = 54) and HIV-1 infected children (n = 38; 25 aviremic and 13 viremic). Panel C shows the correlation between CXCL13 in plasma and HIV-1 RNA copies. Panel D shows antibody levels to HBsAg measured in the blood of healthy controls (n = 54) and HIV-1 infected children (n = 38). The horizontal lines show the median values within the respective groups. The red symbols show the levels of CXC12, CXC13 and antibody levels to HBsAg of HBV vaccine non-responders. T-test and ANOVA were used to calculate the differences between healthy controls and HIV-1 infected children. ns = not statistical significant; *p < 0.05, **p < 0.01, ***p < 0.001; ****p < 0.0001.

Table 3
Correlation of CXCL13 plasma levels with B cell subsets and CXCR5+B cell subsets in healthy controls and HIV-1 infected children.

B cell subsets	B cell subsets vs CXCL13		CXCR5+B cell subsets vs CXCL13	
	HC	HIV-1	HC	HIV-1
Total B cells	n.s.	n.s.	n.s.	r = −0.70 p < 0.0001
Naïve	n.s.	r = −0.42 p = 0.008	n.s.	r = −0.50 p = 0.001
RM	n.s.	n.s.	n.s.	r = −0.51 p = 0.001
AM	n.s.	r = 0.55 p = 0.0004	r = −0.29 p = 0.03	r = −0.39 p = 0.02
TLM	n.s.	r = 0.58 p = 0.0001	n.s.	r = −0.40 p = 0.01
Switched memory	n.s.	r = −0.45 p = 0.005	n.s.	r = −0.69 p < 0.0001

n.s. = not significant.
RM = resting memory B cells; AM = activated memory B cells; TLM = tissue-like memory B cells.

4. Discussion

Our results show that HIV-1 infected children receiving ART carry profound defects in frequencies and homing property of B cell subsets which may directly affect immune responses to vaccines and longevity of memory B cells.

The frequency of RM B cells, cells involved in serological memory to vaccines and pathogens, was lower in ART treated children compared to healthy controls; this difference was mostly observed for virological non-responders suggesting that frequency normalization of RM B cells occurs when HIV-1 replication is successfully suppressed in infected children. On the other hand, the frequency of exhausted TLM B cell subset was high in HIV-1 infected children, independently of viremia control. Previous studies, conducted in African settings, showed that altered frequencies of RM and exhausted memory B cells in HIV-1 infected children are only partially corrected by ART [14,15]; high HIV-1 RNA copies in circulation, due to low ART adherence, could contribute to declined frequencies of RM B cells. Also in our study a significant correlation was found between the frequency of RM B cells and viral load in ART treated children.

When compared to CD27+ RM B cells, CD27-TLM B cells have a reduced capacity to differentiate to antibody-secreting B cells upon stimulation with influenza antigens [3]. GC interaction of CD27 on B cells with CD70 on Tfh cells is critical for maturation and differentiation of B cells; in absence of this interaction, B cell responses to T cell dependent vaccines are impaired [16,17]. Thus, enlarged populations of CD27- exhausted memory B cells during HIV-1 infection might affect responses to childhood vaccines.

The expression of both CXCR4 and CXCR5 chemokine receptors by naïve and RM B cells is pivotal to ensure humoral responses to antigens as, by responding to their respective ligands, B cells will be re-directed to specific structures within the lymphoid tissues where their differentiation can occur. The chemokine receptor CXCR4 is expressed by B cells which are positioned in the GC dark zone, where the expression of their ligand CXCL12 is highly enriched, to undergo somatic hypermutation (SHM) and affinity maturation [18]. We found that the frequency of CXCR4 expressing B cell subsets was significantly reduced in HIV-1 infected children with plasma CXCL12 levels slightly higher in HIV-1 infected children [19]. According to our results, reduced expression of CXCR4 on both naïve and RM B cells may lead to premature release of B cells to GC light zone, resulting in a low degree of SHM and proliferation [20].

In the final phases of differentiation in the GC dark zone, B cells upregulate CXCR5 and migrate from the dark into the light GC zone following a CXCL13 concentration gradient. In CXCR5 deficient mice, B cells were unable to enter the follicles and accumulated in the T cell areas [21]. In our study, the frequency of CXCR5+ naïve B cell subset was significantly lower in HIV-1 infected children. A reduced expression of CXCR5 might lead to impaired B cell entry in the light GC zone, thus affecting the development of specific memory B cells. Reduced CXCR5 expression by B cells may be due to high CXCL13 levels in circulation leading to receptor internalization [12].

As in other studies [13,22], we found elevated CXCL13 plasma levels in HIV-1 infected children, possibly due to monocyte activation by HIV-1 particles [23]. A recent study showed that CXCL13 levels during primary HIV-1 infection predicted the emergence of broad-neutralization antibodies (bNAbs) in ART naïve patients, suggesting that CXCL13 could be a biomarker for GC activity [22]. Havenar-Daughton and colleagues also showed a possible CXCL13 role as a biomarker for GC activities through the association of CXCL13 levels with bNAbs and increased numbers of GC B and Tfh cells [24]. High CXCL13 plasma levels were however reported in various autoimmune conditions of humans [25] and is therefore unclear if high CXCL13 levels have a beneficial role

in promoting protective B cell responses or if they also activate autoreactive B cells. A conflicting role for CXCL13 also emerges from our study as the expression of CXCR5 on B cell subsets was inversely correlated with plasma CXCL13 concentration in HIV-1 infected children. Thus, high CXCL13 plasma level may lead to internalization of CXCR5 expression in B cells impairing the homing capacity of B cell subsets to the GC for induction of vaccine specific memory B cells [12]. In addition, the significant positive correlation observed between AM and TLM B cell populations and CXCL13 concentration in plasma suggests that this chemokine may be linked to B cell exhaustion during HIV-1 infection.

A previous study showed that B cells from HIV-1 infected individuals expressed high levels of CCR6, known to mediate homing to inflamed tissues [11]. In our study, exhausted memory B cells of ART treated children expressed elevated levels of CCR6 which may facilitate their migration to inflamed tissues as previously described in SIV infected macaques [26].

The plasma concentration of anti-HBs antibodies in HIV-1 infected children was positively correlated with RM frequencies and switched memory B cells in circulation. Our results show that an important reason for poor response to HBV vaccine and other childhood vaccinations may reside in the impaired migratory capacity of B cells to and within organs important for their differentiation; addressing this aspect of B cell immunopathology may improve vaccine responses in HIV-1 infected individuals.

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Statement on conflict of interest

The authors do not have an association that might pose a conflict of interest.

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