Qualitative and quantitative HIV antibodies and viral reservoir size characterization in vertically infected children with virological suppression

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TITLE

Qualitative and quantitative HIV antibodies and viral reservoir size characterization in vertically infected children with virologic suppression

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SHORT TITLE

HIV antibodies and reservoir size characterization in children

SYNOPSIS

Background

The absence of detectable viremia after treatment cessation in some vertically HIV-infected (VHIV) children suggests that early initiation of highly active combination of antiretroviral therapy (HAART) could lead to functional cure.

Objectives

We described the factors associated with HIV antibody levels and the viral reservoir size in HAART-treated VHIV children.

Patients and methods

This study included 97 VHIV children with virological suppression, in Bamako, Mali. The anti-gp41 antibody activities and HIV serostatus were assessed. The viral reservoir size was measured by quantifying total cell associated HIV DNA.

Results

Among the children studied, the median total HIV DNA level was 445 copies/10^6 cells (IQR = 187 - 914), the median anti-gp41 antibody activity was 0.29 OD (IQR = 0.18 - 0.75). Low activity of anti-gp41 antibodies was associated with a younger age of HAART initiation ($p = 0.01$). Overall, eight HIV-1 seroreversions were identified.

Conclusion

This study identified potential candidates with low viral reservoir and low antibody levels or activities for future trials aiming to reduce HIV-1 reservoir in order to limit HAART duration.

INTRODUCTION

Pediatric HIV infection remains a major public health issue despite large implementation of prevention of mother-to-child transmission programmes. [1] It requires early and lifelong HAART to control the viral replication with a risk of accumulating toxicity and viral drug resistance. International guidelines are now recommending initiation of HAART in all vertically HIV-1-infected (VHIV) children regardless of clinical and immunological conditions [2] as early treatment reduces mortality and improves immune recovery. [3]

Proviral DNA HIV-1 reservoirs are established early during infection and represent a barrier to functional cure. [4] A low HIV viral reservoir is associated with a lower risk of disease progression. Despite prolonged HAART, HIV-1 persists as transcriptionally inactive proviruses in long half-life memory resting CD4 T cells.[5] The lowest reservoir has been described in elite controllers (EC) in whom HIV-1 replication is controlled without treatment. [6] However, in post-treatment controllers (PTC), HIV-1 remission and low reservoir are observed after HAART interruption mainly when treatment was initiated during primary HIV infection (PHI). [7] The relation between the size of the viral reservoir, and the precocity and duration of HAART has been previously described in children. [8–10] However, more studies are required to identify simple predictors of the reservoir size in VHIV children, in real world conditions within high HIV prevalence settings, especially in VHIV children.

The absence of detectable viremia 12 years after treatment cessation in one VHIV child has suggested that early HAART initiation could lead to functional cure. [11] HIV infected people first develop anti-gp41 antibodies and only several weeks later, anti-gp120 antibodies. [12] The Berlin patient, who was cured of HIV following a stem cell transplant, dysplayed a complete loss of anti-p24 antibodies and a low but still detectable response to gp41. [13] Only few EC subpopulations show this HIV antibody profile. Thus, monitoring the response to p24 and gp41 may be useful in HIV cure studies.

Several HIV-1 seroreversions have been observed in VHIV children who initiated HAART within the first months of life. [14,15] By blocking viral replication, the early virostatic treatment might prevent the development of the HIV-1 specific antibody response, either quantitatively (antibody level) or qualitatively (antibody

activity), thus leading to HIV seroreversion or substantially lower anti-HIV antibody levels. One analysis demonstrated that age of HAART initiation and plasma viral load were strong predictors of serostatus, and both were independently associated. [16] Kuhn et al showed that the absence of HIV antibody response indicated a smaller HIV-1 viral reservoir, and HAART initiated at 3 months of age was the upper limit to see the benefits of early HAART. [17] Only one study has desmontrated the utility of HIV serostatus as a surrogate marker of the reservoir size. [8]

Here we describe the factors associated with HIV antibody activity or level and the viral reservoir size in HAART-treated VHIV children with heterogeneity of age, time of therapy, and duration of virological suppression.

METHODS

This cross-sectional study conducted within a prospective cohort included HAART-treated VHIV children followed at Gabriel Touré Hospital (Bamako, Mali) with sustained virological suppression (HIV-1 RNA plasma ≤ 50 copies/mL). All participants were known virologically suppressed at their previous visit (six months) and confirmed during the study. Participants older than 20 years old, those with HIV-2 infection, treated for less than three months, or without any data recorded were excluded from the study. After obtaining the parent's written or oral informed consent from all participants, five millilitres of extra blood samples were collected in EDTA tubes during the routine follow up visit. Assent was obtained from child participants according to local institutional review board guidelines. The study was approved by the National AIDS program at the Ministry Of Health in Mali (CSLS/MS) in collaboration with the Malian Institutional Ethics Committee at the Faculty of Medicine, Pharmacy and Odontostomatolgy of health and life sciences in Bamako under the reference number N°10-05-FMPOS.

In order to evaluate the size of the HIV-1 viral reservoir, total DNA was extracted from PBMC's derived from whole blood using an automated technique (MagNA Pure, Roche, Manheim, Germany). The cell-associated HIV-1 DNA level was quantified using a real-time PCR method which amplifying a region in the LTR gene, as previously described. [18] Proviral burden was expressed as HIV DNA copies per 1 million cells (quantification limit: 10 copies/PCR, i.e. 66 copies/10^6 cells considered as undetectable).

Dried serum spots (DSS) were used to evaluate the HIV-specific antibody response. The level of antibodies targeting the gp41 immunodominant epitope (IDE) was measured following a previously described protocol. [19] An equimolar mixture of two 30 amino-acids oligopeptides was used, representing the IDE consensus sequences of HIV-1 group M and subtype D, respectively. A low mixture concentration allowed the binding of late antibodies that had acquired sufficient avidity and then semi-quantitative detection by spectrophotometry. The result was expressed as an optical density (OD). The activity of anti-gp41 antibodies was systematically tested in quadruplicate.

The 4th generation ARCHITECT HIV Ag/Ab Combo assay (Abbott Laboratories, Wiesbaden, Germany) was performed as previously described to quantify the humoral response in DSS. The result was defined as relative light units, then compared to a cut-off signal. Samples with signal-to-cut-off (S/CO) values ≥ 1.00 were considered reactive and those < 1.00 non-reactive.

We used univariate association between HIV DNA level, anti-gp41 antibody activities or HIV-antibody levels and variables defining current and past HIV disease (age, sex, WHO stage at study, HAART type at study, CD4 cell count at study, HAART duration, maternal prophylaxis type, age at HAART initiation), with the Spearman rank correlation coefficient for continuous variables and the Fisher's exact test for categorical variables.

RESULTS

From August 2013 to April 2014, 97 VHIV children with virological suppression were enrolled. Their median age was 9.8 years old at time of inclusion (IQR = 7 – 13.1), they started HAART at a median age of 3.3 years (IQR = 1.9 - 7), and were receiving HAART for a median 5.4 years (IQR = 3.5 - 7). Table 1 summarizes the demographic, immunologic and virologic characteristics of the children.

The median anti-gp41 antibodies activity was 0.29 OD (IQR = 0.18 - 0.75). A low activity of anti-gp41 antibodies was associated with both a younger age at treatment initiation (p = 0.01; Figure 1) and with a lower level of anti-HIV antibodies (p = 0.0015; Figure 1). Overall, eight seroreversions were identified (negative ELISA Architect) in which 2 children had an HIV DNA under the threshold (1 detectable and 1 undetectable) and a low anti-gp41 antibodies activity. All the seroreverted children started HAART before two years of age, at a median age of 1.1 years, and were on HAART for the past 7.3 years in median.

The median level of total HIV DNA was 445 copies/10^6 cells (IQR = 187 – 914). No correlation was found between anti-gp41 antibodies activity or age at treatment initiation and HIV DNA (p = 0.27; Figure 1). The 9 children with an HIV DNA level under the threshold tended to have a lower anti-gp41 antibodies activity compared to children with an HIV DNA > 66 copies//10^6 cells (p = 0.11).

DISCUSSION

This study indicates that a significant proportion of virologically suppressed VHIV children who initiated HAART before the age of two years stopped to produce and/or progressively lost the HIV antibodies. This is consistent with the idea that early HAART halts the antigenic stimulation which is necessary to sustain an HIV-specific antibody response. [8-10,20] In addition, some of these children with seroreversion had a very low HIV reservoir, at least identified in the peripheral blood, and could therefore represent an ideal population for studies investigating novel immunotherapeutic strategies aiming to achieve HAART-free remission.

Although current HAART can effectively control HIV replication to clinically undetectable levels for years, existing strategies do not eradicate HIV-1 reservoirs in VHIV children. [11] One of the limitation of our study was that our cohort did not include children who started HAART before five months of life, and therefore we were not able to identify more seroreversions. Nonetheless, we found 50% of seroreversion in VHIV children who initiated treatment before two or one years old (8/16 and 4/8 respectively), consistently with other studies in occidental settings that showed 50% to 94% of seroreversion when treatment was initiated before 3 months of life. [14,15,20]

Children can acquire HIV-1 in utero, during delivery or breastfeeding. [1] In our study, the time of HIV transmission was not known. But, we assumed that seroreversion probably occured in children who had treatment initiation soon after the HIV infection acquisition. Indeed, when using one of the most sensitive assays available, we were able to find several seroreversions. In addition, we found an association between a low anti-gp41 antibodies activity and a younger age at treatment initiation.

Early effective control of HIV replication has been associated with incomplete development of HIV-specific immune responses in children. [14,15,20] It would be of interest to study the initial development of anti-HIV antibodies (like activity and quantification of anti-gp41 antibodies) in early treated infants to determine whether the primary responses is affected or the influence occurs later on, leading to a decrease of responses

over time. The decrease of antibody production and/or their avidity against some epitopes should reflect the absence of circulating antigenic viral particles, showing the absence of residual viral replication.

In conclusion, the results of this study show that HIV-1 seroreversion and low anti-gp41 activity in VHIV children with early HAART initiation happened and should be considered as a proof-of-concept study to evaluate strategies targeting HAART-free remission (i.e long-term undetectable viremia for an undefined period, in the absence of HAART). [21] This study identified potential candidates with low viral reservoir and low antibody levels or activity. Defining the immunologic and virologic end-points after HAART in VHIV children and identifying factors and biomarkers associated with limited proviral reservoir size are essential to define therapeutic strategies, in order to achieve HIV remission or cure in this population.

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TRANSPARENCY DECLARATION

None to declare.

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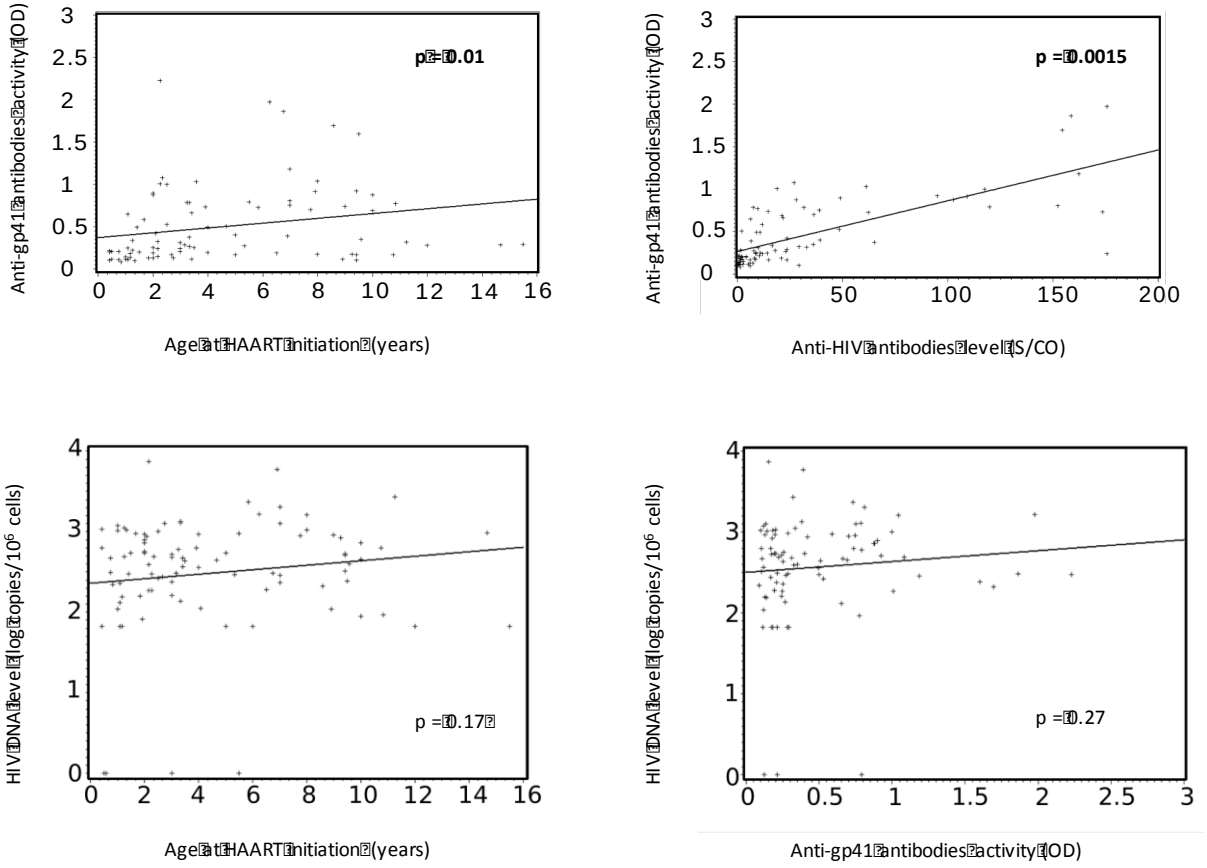
.

Table 1 : Descriptive characteristics of HAART-treated VHIV children with virologic suppression

Abbreviations : cART, combination of antiretroviral therapy; HAART, highly active combination of antiretroviral therapy; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OD, optical density; PI, protease inhibitor; S/CO, signal-to-cutoff; WHO, Word Health Organization

Characteristics	n (%)	Median (IQR)	Global range
Age (years)	-	9.8 (7 - 13.1)	2.8 - 19
Sex :			
Female	38 (39)	-	-
Male	59 (61)	-	-
cART maternal prophylaxis type :			
2 NRTI + 1 NNRTI	60 (62)	-	-
2 NRTI + 1 PI	37 (38)	-	-
Age at HAART initiation (years)	-	3.3 (1.9 - 7)	0.41 - 16
Duration of HAART (years)	-	5.4 (3.5 - 7)	0.33 - 12.1
WHO stage at HAART initiation:			
1 or 2	36 (39)	-	-
3 or 4	52 (61)	-	-
Missing data : 9			
HAART type at study :			
2 NRTI + 1 NNRTI	61 (63)	-	-
2 NRTI + 1 PI	36 (37)	-	-
CD4 cell count at study (cells/mm^3)	-	820 (605 - 1120)	46 - 2000
Missing data : 1			
HIV DNA level at study (copies/10^6cells)	-	445 (87- 902)	0 - 7378
< 66 copies/10^6cells (i.e undetectable)	12 (13)	-	-
Missing data : 2			
Anti-gp41 antibodies activity at study (OD)	-	0.29 (0.18 - 0.75)	0.09 - 2.23
Missing data : 9			
Anti-HIV antibodies level at study (S/CO)	-	14.1 (4.1 - 39.3)	0.31 - 520.6
< 1.0 S/CO (i.e seroconversion)	8 (9)	-	-
Missing data : 10			

Figure 1: Distribution of anti-gp41 antibodies activity by age at HAART initiation (A) and by anti-HIV antibodies level (B) using Spearman correlation. Distribution of HIV DNA level by age at HAART initiation (C) and by anti-gp41 antibodies activity (D) using Spearman correlation.



Abbreviations: HAART, highly active combination of antiretroviral therapy; OD, optical density; S/CO, signal-to-cutoff

Anti-gp41 antibodies activity measured by manual immuno-enzymatic assay

Anti-HIV antibodies level measured by a 4th generation ARCHITECT HIV Ag/Ab Combo assay