Background

It is still unknown whether the paucity of HIV-specific immune responses in early-treated (treated within 6 months of age; ET) HIV-infected children may represent a limitation or an advantage in the perspective of immune therapeutic studies. In ET patients, the failure to develop an immune response is attributed to the lack of persistence such as in ET HIV-infected children, has been poorly investigated. In this study we aimed to investigate ET patients with different HIV Ab profiles and relate those to the predicted size of HIV-reservoir (Total HIV-DNA) and to immune memory B-cell responses.

Methods and Study Cohort

Plasma samples of 15 vertically-infected children followed at “Children’s Hospital Bambino Gesù” in Rome” were tested by fourth-generation Abbott Architect HIV Ag/Ab Combo assay (Chemiluminescent Microparticle Immuno-Assay [COMIA], enzyme-linked immunosorbent assay [ELISA] to 4 different ENV proteins (UG37 gp41, CRF41 gp120, gp41140 and gp120 gp120) and Western blot (WB). Neutralization activity was evaluated with a TZM-bl luciferase assay by incubating patient serum or HIV-specific purified IgGs with 2 HIV pseudoviruses (H962/956.01 c1c8 and Bala2 c8) and VSV-G virus as negative control at different dilution factors. B-cell Fluorospot was performed in polyclonally activated peripheral blood mononuclear cells (PBMCs) to simultaneously detect IgM and IgG memory responses to gp160, gp120, gp41, p24 and p17.

Results

Time of ART Initiation is Associated with Different Serological Profile

Ab responses in 15 ET under stable viral control (undetectable at least 4 years) against 9 HIV proteins (gp120, gp160, gp 41, p24, p17, p51, p45, p39, p35) were analysed by WB. Black and white squares represent presence or absence of Ag-specific ABs. Five of eight ET patients treated within 12 weeks present undetectable antibody response, whereas six out of eight (two under 12 and 24 weeks of age, have detectable antibody response. A WB score was assigned to each patient by adding up the number of positive responses (black squares).

B-cell Fluorospot was performed to simultaneously detect IgM and IgG responses to gp160, gp120, gp41, p24 and p17. Radar plots showed the magnitude of HIV-specific IgM and IgG responses in SNP and SPP.

B-sides Seronegativity: Predominant IgM Memory B-Cell Responses in SNP

A predominant IgM B-cell memory response to HIV-Ags (gp160, p120, gp41, p24 and p17) was observed in the SNP compared to age matched SPP. No significant differences were observed in IgG responses among the two groups. No Ig neither IgM memory responses were observed in the healthy control group.

Conclusions

• Time of ART initiation directly affects the size of viral reservoir. The correlation found between profiles of AB responses and HIV-DNA suggests that WB score could predict the HIV reservoir size (low/positive WB score= small HIV reservoir).
• Seronegative patients present a predominant IgM B-cell memory responses after in vitro stimulation with HIV antigens, suggesting the absent of long term memory responses without a new antigenic stimulation.
• Overall, no neutralization activity was observed in ET children confirming as most likely this population requires immunotherapy in order to mount an effective HIV specific immune response.

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