Negative HIV Serology in Children Treated Early Reflects a Unique Immunologic Profile

1038

Florence Buseyne[1], Caroline Charre[2], Josiane Warszawski[3], Jerôme Le Chenadec[3], Thomas Montange[1], Ingrid Fert[1], Adeline Mélard[2], Elise Gardiennet[2], Stéphane Blanche[4], Catherine Dollfus[5], Marie-Dominique Tabone[5], Albert Faye[6], Pierre Frange[4], Véronique Avettand-Fenoel[2,7], and the ANRS-MIE EP59-CLEAC Study Group

[1]Institut Pasteur, Université Paris Cité, Paris [2]INSERM U1016, CNRS UMR8104, Université Paris Cité, Institut Cochin, Paris [3]INSERM U1018, Université Paris Saclay, Le Kremlin Bicêtre [4]APHP, Hôpital Necker, Université Paris Cité, Paris [5]AP-HP, Hôpital Trousseau, Paris [6]AP-HP, Hôpital Robert Debré, Paris [7]CHU d'Orléans, France

BACKGROUND

Negative HIV serology in children living with HIV and treated early is thought to result from low exposure of the immune system to antigenic stimulation provided by HIV replication. This serological profile might be used to identify pediatric patients eligible for HIV cure strategies. We aimed to characterize parameters associated with negative HIV serology in children who initiated ART early.

METHODS

The presence of HIV-1 antibodies was determined using the EIA VIDAS HIV DUO ULTRA test (Biomérieux). Blood lymphocytes were quantified by flow cytometry. PBMCs were stimulated by a TLR7/8 agonist and IL-2, cultured before testing plasmablast expansion by flow cytometry at day 7 and HIV-specific antibody secretion in an ELISA assay at day 12. The total cell-associated HIV DNA was quantified using ultrasensitive real-time PCR (Biocentric, Bandol, France) in PBMCs. HIV-1 p24 antigen was quantified in plasma samples by the ultrasensitive Quanterix Simoa p24 assay. Fisher and Mann-Whitney tests were used to analyze variables associated with negative HIV serology.

PARTICIPANTS

The analysis included 17 children living with HIV, aged 5-12 yrs, from the French ANRS-EP59-CLEAC study [a,b] who:

- initiated cART before six months of age,
- reached sustained virological success (HIV-RNA < 400 copies/mL in the 24 months after cART initiation and no subsequent rebound),
- and had HIV RNA < 50 copies/mL at the time of the study.

Variable		n (%) median[IQR]
Sex Male		4 (23.5)
Female		13 (76.5)
Sub-Saharan Africa origin	No	7 (41.2)
	Yes	10 (58.8)
Age, years		9 [6;11]
CD4 count, cells/µL		1090 [787;1370]
CD4/CD8 ratio		1.74 [1.37;1.88]

AUTHOR CONTACT
florence.buseyne@pasteur.fr

In children aged 5-12 yrs with limited exposure to HIV replication,
Negative HIV Serology was:
- frequent (41% of participants);
- associated with a higher % of a CD8 T cell subset with high antiviral activity;
- not related to a memory B cell defect.

Negative HIV Serology may predict immune control in future HIV cure strategies.

RESULTS

- Seven participants had negative HIV serology (seronegative, SN, 41%) and 10 had positive HIV serology (seropositive, SP, 59%).
- SN children tended to be younger at cART initiation than SP children (14 vs 97 days, $P = 0.06$, FIG1A). At the time of the study, surrogate markers of past HIV exposure did not distinguish SN from SP children (total HIV DNA, 1.9 [1.4; 2.2] vs. 2.2 [1.2; 2.5] $\log_{10}$ copies/10^6 PBMCs, $P = 0.64$, FIG1B; plasma HIV-1 p24 detection: 25% vs 38%, $P = 0.67$).

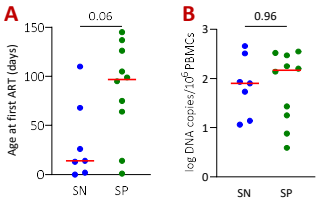


FIGURE 1. (**A**) Age at first cART initiation and (**B**) HIV DNA levels at the time of the study are presented. *P* values from the Mann-Whitney test are presented

- B and circulating follicular CD4 T lymphocytes levels were similar in both groups (FIG2A-B).
- SN children had a higher percentage of circulating follicular $CXCR5^+CCR4^-$ CD8 T_{EM} lymphocytes than SP children (3.1% [2.0; 3.2] vs 1.3% [0.7; 2.0], $P = 0.01$, FIG2C).
- Although rarely detected, HIV-specific B cells were present in both SN and SP children after *in vitro* expansion (FIG2D-F).

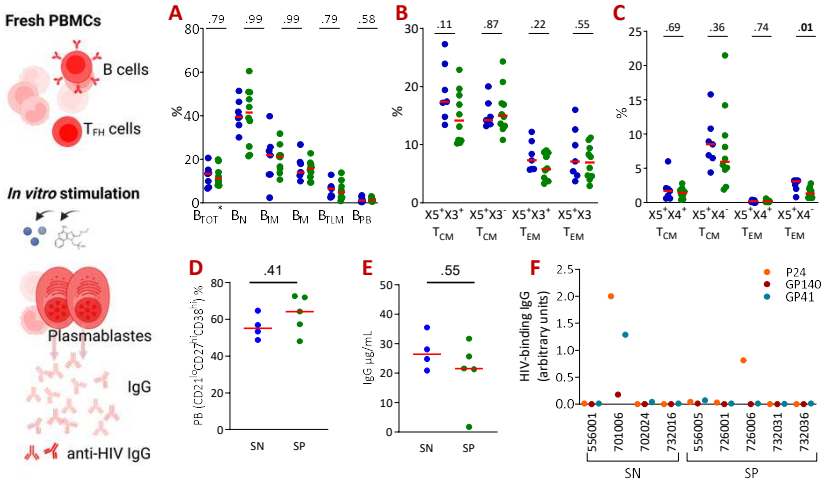


FIGURE 2. (**A**) Major B lymphocytes subsets were quantified on fresh blood (N : $CD10^-CD27^-$ naïve; IM $CD10^+CD27^-$ immature; M; $CD21^+CD27^+$ memory, TLM : $CD21^{lo}CD27^+$ tissue like memory; PB: $CD20^-CD27^+CD38^{hi}$ plasmablasts. (**B**) Major CD4 $CXCR5^+$ T_{FH} subsets defined by CXCR3 coexpression and differentiation stage (T_{CM}, central memory, T_{EM} effector memory) (**C**) Major CD8 $CXCR5^+$ T_{FH} subsets defined by CCR4 coexpression and differentiation stage. PBMCs were stimulated with R848 and IL-2 and cultured before the quantification of (**D**) PB expansion; (**E**) total and (**F**) HIV-specific IgG secretion into supernatants. *P* values from the Mann-Withney test are presented

CONCLUSIONS

Among children over the age of five years with limited exposure to HIV replication, negative HIV serology could not be attributed to B lymphocyte defects. Rather, it was associated with expanded circulating follicular CD8 T lymphocytes, previously reported to have high antiviral activity[c,d]. Thus, negative HIV serology may be a biomarker of a good potential for HIV immune control in the context of future HIV cure strategies.

REFERENCES
[a]Avettand-Fenoel, V, et al., (2021) Clin. Infect. Dis. 73, e4214-e22, PMID:34355738.
[b]Frange, P, et al., (2021) Front. Immunol. 12, 662894, PMID:33968064.
[c]Perdomo-Celis, FMD, et al., (2018) JAIDS 78, 473-82, PMID:29649077.
[d]Collins, DR, et al., (2023) Science Immunol 8, eade5872, PMID:37205767.

ACKNOWLEDGMENTS: We thank all the patients, their families, the clinical investigators (M.F. Courcoux, (Trousseau Hospital, Paris), C. Fourcade (Bicêtre Hospital, Le Kremlin Bicêtre), I. Hau (Centre Hospitalier Intercommunal, Créteil), C. Gakobwa (Delafontaine Hospital, Saint Denis), N. Mahlaoui, F. Veber (Necker – Enfants malades Hospital, Paris), A. Chacé (Centre Hospitalier Intercommunal, Villeneuve Saint Georges)) and the members of the ANRS CLEAC study group (D. Scott-Algara, A. Saez-Cirion (Pasteur Institute, Paris), B. Autran and A. Samri (Pitié Salpêtrière Hospital, Paris), S. Caillat-Zucman (Saint Louis Hospital, Paris), O. Dialla, L. Ait Si Selmi, I. Leymarie, T. Wack and L. Boufassa (INSERM, Le Kremlin Bicêtre), C. Floch-Tudal (Louis Mourier Hospital, Colombes), E. Lachassine (Jean Verdier Hospital, Bondy), A. Diallo, L. Marchand, C. Paul and V. Petrov-Sanchez (ANRS), H. Pollard (TRT-5)). We thank the logistic platform from Institut Pasteur in Paris, S. Novault and P.H. Commere from Utechs Cytometry and Biomarkers from Institut Pasteur Paris. We thank the nurses and staff from clinical sites, S. Leveillé, MC. Mourey, G. Vaudre and M. Vassal.