Original article

Early therapy in HIV-1-infected children: effect on HIV-1 dynamics and HIV-1-specific immune response

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Background: Perinatal HIV-1 infection is acquired in the milieu of a developing immune system, leading to high levels of uncontrolled viral replication. Few data have been reported that address the viral dynamics and immunological response in infants who initiated aggressive antiretroviral therapy (ART) shortly after birth.
Methods: Six HIV-1-infected infants who started ART within 3 months of age were studied. The median follow-up was 61 months. Plasma HIV-1 RNA, cell-associated HIV-1 DNA, unspliced and multiply spliced HIV-1 mRNAs, HIV-1 antibodies, and CD4$^+$ and CD8$^+$ T-cell subsets were assessed in sequential peripheral blood samples. HIV-1 cellular immune response was measured by EliSpot assay.
Results: All children showed a decline in plasma viraemia to undetectable levels. HIV-1 DNA persisted in four children, but only two of these had detectable HIV-1 mRNA. All viral parameters remained persistently negative in two children. Only two children produced HIV-1 antibodies, while the others, after having lost maternal antibodies, remained seronegative. No HIV-1 cellular immune response was observed in any child. Therapy interruption was performed in two children: one HIV-1-seropositive and one HIV-1-seronegative with persistently undetectable levels of all viral parameters. Rebound of HIV-1 plasma viraemia in the seronegative child was more rapid and higher than that observed in the seropositive child.
Conclusions: Early ART treatment in infants modifies the natural course of infection by controlling HIV-1 replication and reducing viral load to below the threshold levels required for onset of HIV-1 immune response, but does not prevent the establishment of a reservoir of latently infected cells that precludes virus eradication.

Introduction

The introduction of antiretroviral therapy (ART) has heralded important changes in the natural course of HIV-1 infection both in adults and children, yet ART is unable to fully suppress HIV-1 replication in chronically infected subjects. Intracellular HIV-1 mRNA and replication-competent virus can be recovered from cells, even in patients with a long period of undetectable HIV-1 RNA in plasma [1–3]. This persistence of latently infected cells prohibits eradication of the infection [4,5].

A body of evidence has demonstrated that HIV-1 replication plays a central role in the pathogenesis of HIV-1 disease [6], and several studies have suggested that the dynamics of HIV-1 during primary infection is predictive of the subsequent disease outcome both in adults and children [7,8]. Studies in adults have shown that treatment of primary infection with ART resulted in a greater and more rapid reduction in HIV-1 plasma viraemia compared with untreated patients [9], as well as decreasing the cell-associated HIV-1 DNA load more efficiently than when ART was initiated during the chronic phase of infection [10]. Although unable to eradicate infection, ART administration during primary infection – by restricting viral replication and preserving immune function – limits the rate of disease progression. Furthermore, early ART initiation can enhance viral control after ART discontinuation.

Although depletion of CD4$^+$ T-cells and disease progression occur more rapidly in children than in adults, few studies have been performed to evaluate the effect of early ART in infants. In one study early ART treatment was associated with good clinical and

immunological outcomes after 72 weeks of follow up, but a high rate of virological failure (that is, plasma HIV-1 RNA never <400 copies/ml or rebounded to >400 copies/ml at 72 weeks) was reported [11]. In another study, ART initiation prior to 3 months of age was associated with a longer period of viral suppression in plasma compared with that observed in infants who had received ART after 3 months of age [12]. Data concerning intracellular HIV-1 dynamics and HIV-1-specific immune responses in early ART-treated infants are very scarce. In the few infants studied, suppression of HIV-1 RNA plasma viraemia for 1–2 years was associated with a lack of virus isolation from the peripheral blood compartment [13] and a lack of HIV-1-specific immune responses [13,14].

In this study, we investigated the intra- and extracellular HIV-1 infection profile and the HIV-1-specific immune responses in long-term treated children who had received ART from the first few months of life.

Methods

Patients

This study included six HIV-1-infected children, born to HIV-1-seropositive mothers who had not received antiretroviral prophylaxis, and followed at the Paediatric Department of Padova University, Italy. The infectious status of the infants was defined by virus isolation and the polymerase chain reaction (PCR) assay performed as previously reported [15]. The inclusion criterion was the initiation of highly active ART within 3 months of age. $CD4^+$ and $CD8^+$ T-cell counts, anti-HIV-1 antibodies and plasma HIV-1 RNA levels were followed over time. Levels of proviral HIV-1 DNA and cell-associated HIV-1 mRNAs were evaluated in cryo-preserved samples.

Quantification of HIV-1 RNA in plasma

Plasma HIV-1 RNA levels were determined by reverse transcriptase-PCR (Roche Amplicor Monitor System, New Jersey, USA) according to the manufacturer's instructions, with a lower limit of detection of 400 HIV-1 RNA copies/ml when using the standard protocol and a detection limit of 50 HIV-1 RNA copies/ml when using the ultrasensitive protocol.

Quantification of HIV-1 mRNAs in cells

Levels of cell-associated HIV-1 RNAs were quantified by real-time PCR, as previously described [3]. Briefly, 1 μg RNA extracted from cells ($1–5\times10^6$) was retrotranscribed using the TaqMan Reverse Transcription reagents assay (PE Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Unspliced HIV-1 mRNAs (HIV-1 RNAus) and multiply spliced HIV-1 mRNAs (HIV-1 RNAms), were quantified by real-time PCR using the primers US1 forward (*5′*-TTAAGTGTTTCAATTGTGGCAAAGA-*3′*; nt 1956–1981) and US2 reverse (*5′*-AAAAAATTAGC-CTGTCTCTCAGTACAATCT-*3′*; nt 2059–2122) and MS1 forward (*5′*-AAAGGGAAACCAGAGGAGCTCT-*3′*; nt 672–693) and MS2 reverse (*5′* GCCT-GTCGGGTCCCCTC-*3′*; nt 8438–8454), and the probes USP (FAM-*5′*-CCCCTAGGAAAAAGGGCT-GTTGGAAATG-*3′*-TAMRA; nt 2007–2035) and MSP (FAM–*5′*-TCGACGCAGGACTCGGCTTGC-*3′*TAMRA; nt 695–715) (HIVPV22 genome, GenBank accession code K02083). The HIV-1 mRNA copies were expressed relative to 10^6 copies glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [3].

HIV-1 DNA quantification

Proviral HIV-1 DNA copies per 10^5 peripheral blood mononuclear cells (PBMC) were quantified by real-time PCR, as previously described [16].

Viral phenotype analyses

Primary isolates were obtained by co-culture of PBMCs from each child with phytohaemoagglutinin (PHA)-stimulated PBMC from healthy donors, as previously described [17]. Viral phenotype analysis was performed in U87MG-CCR5 and U87MG-CXCR4 cell lines that stably expressed CD4 and CCR5 or CXCR4 coreceptor [18]. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 50 μg/ml gentamicin, 2% glutamine and 10% fetal calf serum (FCS). For infection assays, cells were seeded in 48-well plates (Falcon, Grenoble, France) at a concentration of 5×10^3 cells/well for 24 h before infection. The cells were washed twice with fresh medium and then exposed to viral isolate (10,000 pg of p24 protein equivalent/well) in a final volume of 0.5 ml. The supernatants were harvested 8 days after infection, and the amount of HIV-1 p24 antigen was quantified using a commercial assay (Vironostika HIV-1 Antigen, Biomérieux, Rome, Italy). In agreement with the proposed classification [19], the viruses were typed as R5, X4, or R5X4 according to their coreceptor usage.

HIV-1 genotyping analyses

Analyses of protease (PR) and reverse transcriptase (RT) sequences of the HIV-1 *pol* gene were performed. HIV-1 DNA was obtained by lysis of 1×10^6 PBMCs, as previously described [8], while HIV-1 RNA was extracted from plasma and PBMC samples using a commercial kit (Qiagen GmbH, Hilden, Germany). Extracted RNA was retrotranscribed using Superscript™ III (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A nested-PCR amplification was performed using the followed primer pairs: outer PR primers

PR1-forward (5′-GATAGACAAGGAACTGTATC-CTTTA-3′; nt 2268–2292) and PR2-reverse (5′-ATCCATTCCTGGCTTTAATTTTACT-3′; nt 2645–2621), inner PR primers PR3-forward (5′-CTTCCCTCAGATCACTCTTT-3′; nt 2294–2313) and PR4-reverse (5′-TGGCTTTAATTTTACTGGTA-3′; nt 2636–2617), outer RT primers RT1-forward (5′-GGACCTACACCTGTCAACATAATTGGAA-GAAA-3′; nt 2529–2560) and RT2-reverse (5′-TTGACAGTCCAGCTGTCTTTTTCTGGCAG-3′; nt 3358–3330), inner RT primers RT6-FW forward (5′-GACAGAAGAAAAAATAAAAGCATT-3′; nt 2672–2695), RT6-RV reverse (5′-TGGAAGCA-CATTGTACTGATATCT-3′; nt 3044–3021), RT4-FW forward (5′-TTCAGGAAGTATACTGCATTTACC-3′; nt 2964–2987) and RT4-RV reverse (5′-TCTTTTTCTGGCAGCACTATAGGC-3′; nt 3343–3320) (HIVPV22 genome, GenBank accession code K02083) [20]. Amplification was carried out in a thermal cycler (9600, Perkin Elmer, Walthman, MA, USA) for 45 cycles, each of 30 s at 94°C, 30 s at 60°C and 1 min at 72°C for PR1–PR2 and RT1–RT2 primers; 30 s at 94°C, 30 s at 54°C and 1 min at 72°C for PR3–PR4 primers; and 30 s at 94°C, 30 s at 58°C and 1 min at 72°C for RT6-FW–RT6-RV and RT4-FW–RT4-RV primers.

PCR products were purified using a commercial assay (ExoSAP-IT, Amersham Biosciences, Buckinghamshire, UK) and directly sequenced using the automatic sequencer ABI PRISM 3130*xl* Genetic Analyzer (PE Applied Biosystems) and the Big Dye Terminator v1.1 cycle sequencing ready reaction kit (PE Applied Biosystems), according to the manufacturer's instructions. Sequences were analysed with Sequencing Analysis Software v.5.2 (PE Applied Biosystems) and aligned to HIVPV22 using SeqScape Software v.2.5 (PE Applied Biosystems). The HIV Drug Resistance Database (http://hivdb.stanford.edu) [21] was utilized to discriminate reverse transcriptase and protease mutations with a major role in conferring high or moderate level of resistance to antiretroviral drugs.

HIV-1 serology

Plasma HIV-1 antibodies were detected using a commercial enzyme-linked immunosorbent assay (ELISA; Enzygnost, Bhering, Marburg, Germany) and by western blotting (HIV blot 2.2, Alfa Wasserman, Bologna, Italy).

In vitro antibody production

Spontaneous *in vitro* antibody production (IVAP) was tested using the standardized protocol [22]. Briefly, PBMC at a concentration of 2×10^6 cells/ml were cultured in RPMI medium supplemented with 10% FCS, 1% L-glutamine, 1% non-essential amino acids, and 2×10^{-5} M 2-mercaptoethanol in the presence or absence of pokeweed mitogen (PWM; 1/100 final dilution). Cell-free supernatants were then recovered by low-speed centrifugation, and total immunoglobulin (Ig) and HIV-1-specific antibody in culture supernatants were measured by solid-phase radioimmunoassay as previously reported [23].

EliSpot assay

HIV-1-specific responses of $CD8^+$ T-cells were detected by EliSpot assay, performed as previously described [24]. Briefly, purified $CD8^+$ T-cells were plated in duplicate at 5×10^4 cells/well in 96-well nitrocellulose-baked plates (MAHA S4510; Millipore, Bedford, MA, USA) precoated with 5 μg/ml of mouse anti-human interferon-γ-monoclonal antibody (Pharmigen, San Diego, CA USA), and incubated with irradiated (5,000 rads) autologous $CD8^+$-depleted PBMC (2×10^5 cells/well), used as antigen-presenting cells, and pulsed or not with the HIV-1 HLA-A2-binding peptides [24] were employed at a final concentration of 10 μg/ml. After 6 h incubation at 37°C, plates were washed and 100 μl of 2 μg/ml biotinylated mouse anti-human interferon-γ (Pharmigen) were added to each well. After 2 h incubation at room temperature and washing, 50 μl of streptavidin horseradish peroxidase (1:500 dilution) were added to each well and plates were incubated for a further 90 min at room temperature. The spots were quantified using the AID EliSpot Reader (AID GmbH, Strassberg, Germany). Results are reported as spot-forming cells (SFC) per 10^6 cells after subtraction of the background response. All peptides employed in this study have been previously described in detail [24].

Results

Characteristics of the study population

This study focused on six HIV-1 infected infants who started ART prior to 3 months of age. The immunological and virological characteristics at baseline are shown in Table 1. Median age at start of ART was 2.75 months (range 2–3 months). At ART entry, the median $CD4^+$ T-cell count was 1,827 cells/μl (range 969–2,880); only one child had a $CD4^+$ T-cell percentage <25%. Median HIV-1 RNA level in plasma was 6.0 (range 4.8–6.0) $\log_{10}$ copies/ml. Virus isolation was performed in three cases and all three primary isolates were found to employ the CCR5 coreceptor. All infants were asymptomatic and initiated triple therapy, including two non-nucleoside reverse transcriptase inhibitors in combination with one protease inhibitor or one nucleoside reverse transcriptase inhibitor (Table 1). The median follow-up period was 61 months (range 40–87 months). Four children

remained on the initial triple drug therapy for the entire follow-up period, while two (EA05 and EA06) had therapy interruption (Table 2).

Virological and immunological changes during ART

Following the initiation of ART, HIV-1 RNA levels in plasma fell below 400 copies/ml after a median time of 17.5 weeks (range 7–30 weeks) and below 50 copies/ml after a median time of 31 weeks (range 20–40 weeks). Thereafter, and before therapy interruption, intensive follow up of these children indicated that plasma HIV-1 RNA remained mainly below detectable levels in all children. A few blips of HIV-1 RNA were observed in four infants, but all were <2,000 copies/ml of plasma (Table 2). To better investigate the extent to which viral replication was controlled, the levels of HIV-1 DNA and HIV-1 intracellular mRNAs were evaluated. The cellular HIV-1 DNA levels remained detectable for the entire follow-up period in 4/6 children (Figure 1A). In three children, HIV-1 DNA levels were measured at the initiation of ART and, after the first month, HIV-1 DNA decayed by 0.70 $\log_{10}$ copies/1×10^5 in subject EA04, by 0.14 $\log_{10}$ copies/1×10^5 in subject EA01 and by 0.06 $\log_{10}$ copies/1×10^5 in subject EA06. The persistence of proviral DNA and the blips of plasma viraemia suggested a persistence of residual viral replication. To explore this possibility, intracellular levels of HIV-1 mRNAs were investigated. HIV-1 RNAus and HIV-1 RNAms decayed rapidly after ART inititation; thereafter, they were repeatedly detected in two children (EA01 and EA06), while only one blip of HIV-1 RNAus was detected in child EA02. Notably, two children (EA04 and EA05) remained persistently negative for all HIV-1 parameters.

The percentage of $CD4^+$ T-cells in peripheral blood at baseline was normal for age and <25% in only one child (EA03). After ART initiation, the percentage of $CD4^+$ T-cells and total $CD4^+$ and $CD8^+$ T-cell counts remained within the normal range for age [25] in all infants (Figure 1B).

HIV-1 immune response

Only two children (EA01 and EA06) remained seropositive, whereas the others lost HIV-1-specific maternal antibodies in a median time of 13 months (range 12–14 months; Table 2) and thereafter remained persistently seronegative (Figure 1B). The clearance of HIV-1-specific antibodies was similar to that observed in uninfected children born to HIV-1-seropositive mothers [26,27], indicating the lack of autochthonous HIV-1-specific antibody production;

Table 1. Characteristics of the study population at baseline

Patient	$CD4^+$ T-cell count, cells/μl (%)	Plasma HIV-1 RNA, $\log_{10}$ copies/ml	HIV-1 primary isolate phenotype	Age at ART entry, months	Antiretroviral therapy
EA01	2,880 (32)	6.0	R5	3	AZT, 3TC, NVP
EA02	1,361 (27)	5.3	ND	2	d4T, 3TC, NVP
EA03	969 (16)	4.8	ND	2	AZT, 3TC, NVP
EA04	1,950 (32)	6.0	R5	2.5	d4T, ddI, NFV
EA05	1,872 (36)	6.0	ND	3	AZT, 3TC, NFV
EA06	1,783 (33)	6.0	R5	3	d4T, ddI, NFV

AZT, zidovudine; ddI, didanosine; d4T, stavudine; ND, not determined; NFV, nelfinavir; NVP, nevirapine; 3TC, lamivudine.

Table 2. Follow up of ART-treated children

Patient	HIV-1 RNA <400 copies/ml, weeks	RNA <50 copies/ml, weeks	Time to seroreversion, months	Blips of HIV-1 RNA, copies/ml	Length of follow up, months	Length of follow-up before TI, months
EA01	10	20	–	95/261/1,580	49	–
EA02	7	30	13	94/86	56	–
EA03	30	40	14	130/119	40	–
EA04	28	32	12	–	66	–
EA05	8	22	13	–	87	49
EA06	25	33	–	159/135/557/932	70	56

TI, therapy interruption.

this was confirmed in one child by the IVAP assay. Although *in vitro* synthesis of total Ig was observed in both untreated and PWM-treated cells (0.201 μg/ml and 0.513 μg/ml, respectively), the production of HIV-1-specific Ig was below the detection limit [23]. After a median time of 37 months (range 33–49 months) from therapy initiation, HIV-1-specific $CD8^+$ T-cell responses were analysed in five $HLA\text{-}A2^+$ children using the EliSpot assay after therapy initiation (range 33–49 months). As shown in Table 3, one seronegative (EA02) and one seropositive child (EA06) did not show $CD8^+$ T-cell responses to any of the 16 HIV-1-derived peptides examined. Two seronegative children (EA03 and EA05) and a seropositive child (EA01) showed a low response against few of the 16 tested HIV-1 peptides.

Therapy interruption

Therapy interruption was performed in one child (EA06) positive for HIV-1 DNA, HIV-1 mRNAs and HIV-1-specific antibodies and in one child (EA05) negative for HIV-1-specific immunity and with persistently undetectable levels of all viral parameters. In the EA06 seropositive child, rebound of plasma viraemia reached a peak of 5.18 $\log_{10}$ copies/ml 12 weeks after therapy interruption and then persistently remained at 4.50 $\log_{10}$ copies/ml during therapy interruption. Plasma viral rebound was preceded by an increase in proviral HIV-1 DNA and HIV-1 RNAus (Figure 2A). In the EA05 seronegative child rebound of plasma viraemia was more rapid and higher than that observed in child EA06, reaching a peak of 5.90 $\log_{10}$ copies/ml 4 weeks after therapy interruption. Of interest, this plasma viral rebound preceded an increase in HIV-1 DNA (peak of 87 copies/10^5 PBMC), HIV-1 RNAus (peak of 1,242 copies/10^6 copies GAPDH) and HIV-1 RNAms (peak of 820 copies/10^6 copies GAPDH) in PBMCS (Figure 2A). Because of this rapid and high rebound of plasma viraemia, as well as the other cell-associated parameters, the therapy was immediately recommenced.

Sequence analyses disclosed no drug resistance mutations in viruses isolated from cells and plasma (data not shown). During therapy interruption, there was a slight decrease of $CD4^+$ T-cells both in child EA05 (from 1,388 to 775 cells/μl) and in child EA06 (from 1,708 to 1,071 cells/μl), but $CD4^+$ T-cell percentages remained consistently >25%. No variations in $CD8^+$ T-cells were observed (data not shown). Both EA06 and EA05 were re-analysed for HIV-1-specific cellular immune response at 1 month and 6 months, respectively, after therapy re-initiation. As shown in Figure 2B, only child EA06 showed a cellular immune response against several HIV-1-derived peptides, while child EA05 remained negative.

Discussion

Perinatal HIV-1 infection is acquired in the milieu of a developing immune system, leading to high levels of uncontrolled viral replication; thus, infants experience rapid disease progression in the absence of ART [8,28,29]. A very early diagnosis and treatment during primary infection should decrease the magnitude of virus dissemination throughout the body, preserve immune functions, and thus reduce risk of clinical progression. However, the effect of early ART on the progression of perinatal HIV-1 infection was not well defined.

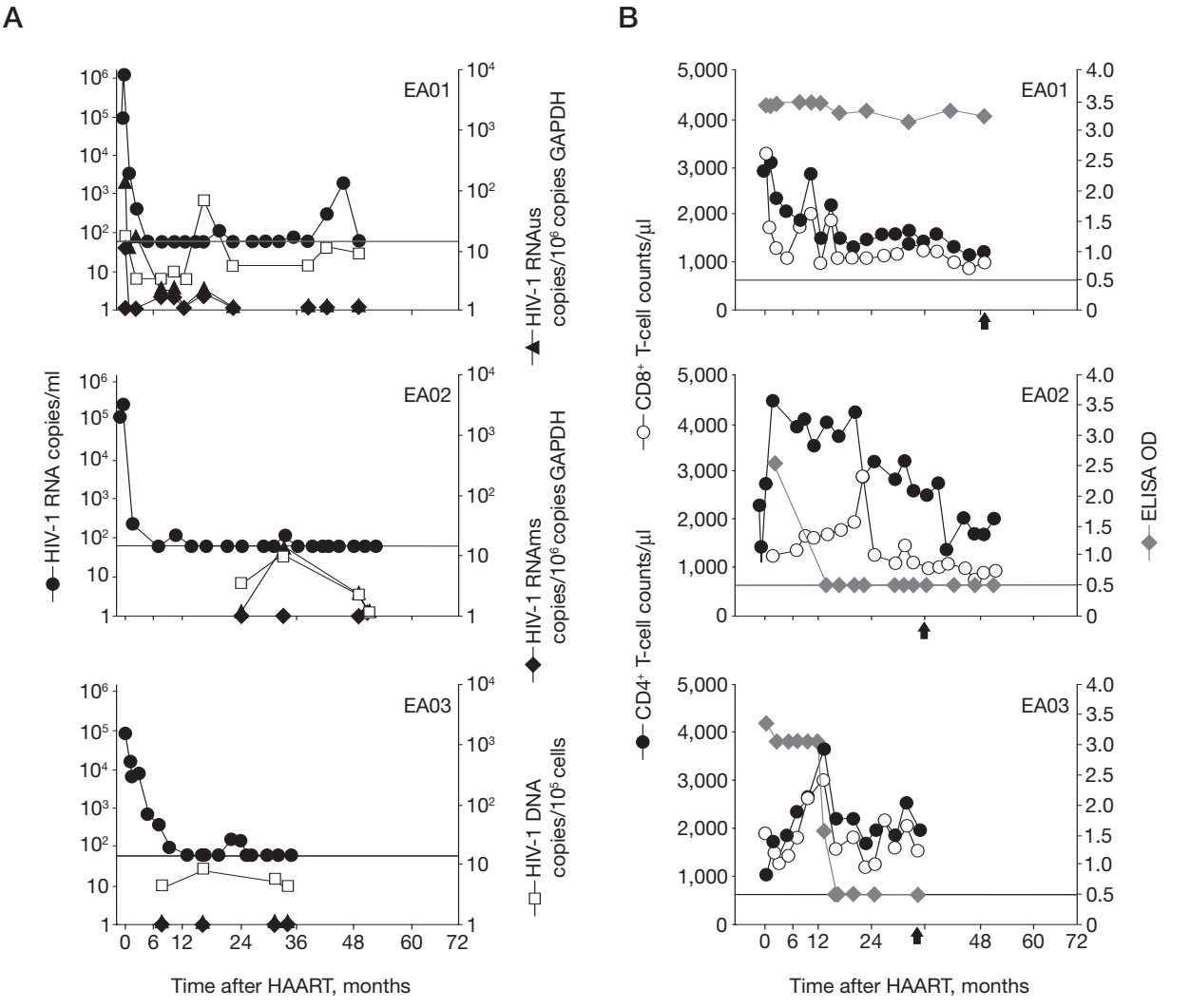
This study investigated the viral dynamics and immune responses of infants in whom antiretroviral therapy was initiated within the first 3 months of life. All infants were virological responders to therapy and, after the initial HIV-1 decline, plasma viraemia remained below the detection limits except for a few blips during the entire long-term follow-up period. This is in agreement with a previous observation that initiation of ART in the first three months of life resulted in improved long-term viral suppression [12]. Despite the fact that children were persistently aviraemic, HIV-1 DNA was consistently detected in the PBMCs of 4/6 children. This persistence may have originated from latently infected cells with a long half-life and/or newly infected cells [30,31]. Four of six children were persistently negative for markers of viral replication, HIV-1 RNAus and HIV-1 RNAms; nevertheless, this finding cannot exclude the presence of residual viral replication activity below the limits of detection.

Of interest, only the two children with detectable viral mRNAs remained seropositive, while the others did not develop autochthonous HIV-1 antibodies and, after having lost maternal antibodies, remained HIV-1 seronegative. This finding extends previous observations [13,14]. In addition, we found that all children were negative for $CD8^+$ T-cell responses against the majority of tested HIV-1 epitopes. The use of only HLA-A2-restricted peptides might have limited the detection of a $CD8^+$ T-cell response; nevertheless, this approach allowed us to perform the analysis on 5/6 infants in our cohort. A recent report indicated that in adults early ART during primary infection was associated with long-term viral suppression, and in a few cases with incomplete humoural response to HIV-1 or seroreversion, probably due to the absence of ongoing antigenic stimulation required for maintaining HIV-1-specific humoural responses [32]. If the timing of treatment initiation is crucial to preserving the immune system in adults, the success of early viral suppression in infants who are immature immunologically is under debate [33]. The absence of HIV-1-specific humoural and cellular immune

responses in this study in early-treated children could reflect a lack of the appropriate (for duration and level) antigenic stimulus required by the developing immune system [34]. With this concern, a previous study performed on children who had commenced ART after 1 year of life indicated that the total $CD8^+$ T-cell response was related to levels of plasma viraemia, and was greatest when viral load was 10^3–10^4 copies/ml plasma. In contrast, in the few studied children who initiated ART within 3 months of life, $CD8^+$ T-cell response was very poor, regardless of plasma viraemia levels [35]. This finding suggests that ART initiation in the first months of life might alter the viral threshold required for the engagement of HIV-1-specific immune responses [35,36].

Analysis of the kinetics and magnitude of viral rebound in plasma and PBMCs after therapy interruption demonstrated a different response in the seropositive and seronegative child. In the seropositive child EA06 there was a rapid rebound of HIV-1 DNA and RNAus, indicating persistence in the peripheral blood compartment of infected cells where the virus had undergone replication immediately after therapy interruption. In the seronegative child EA05, the rebound of plasma

Figure 1. Virological and immunological profiles of infants during early antiretroviral therapy



(A) The kinetics of plasma HIV-1 RNA, HIV-1 DNA, HIV-1 unspliced RNAs (RNAus), and HIV-1 multiply spliced RNAs (RNAms) determined by real-time PCR in peripheral blood mononuclear cells (PBMC). The horizontal line represents the lower limit of detection of the assays for plasma HIV-1 RNA. **(B)** $CD4^+$ and $CD8^+$ T-cell counts and HIV-1-specific antibody levels determined by ELISA. The identification codes of patients are reported within each panel. The horizontal line represents the lower limit of detection of the assays for HIV-1-specific antibodies. The back arrow indicates the time point when the EliSpot assay was performed. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAART, highly active antiretroviral therapy; OD, optical density.

Figure 1. Continued

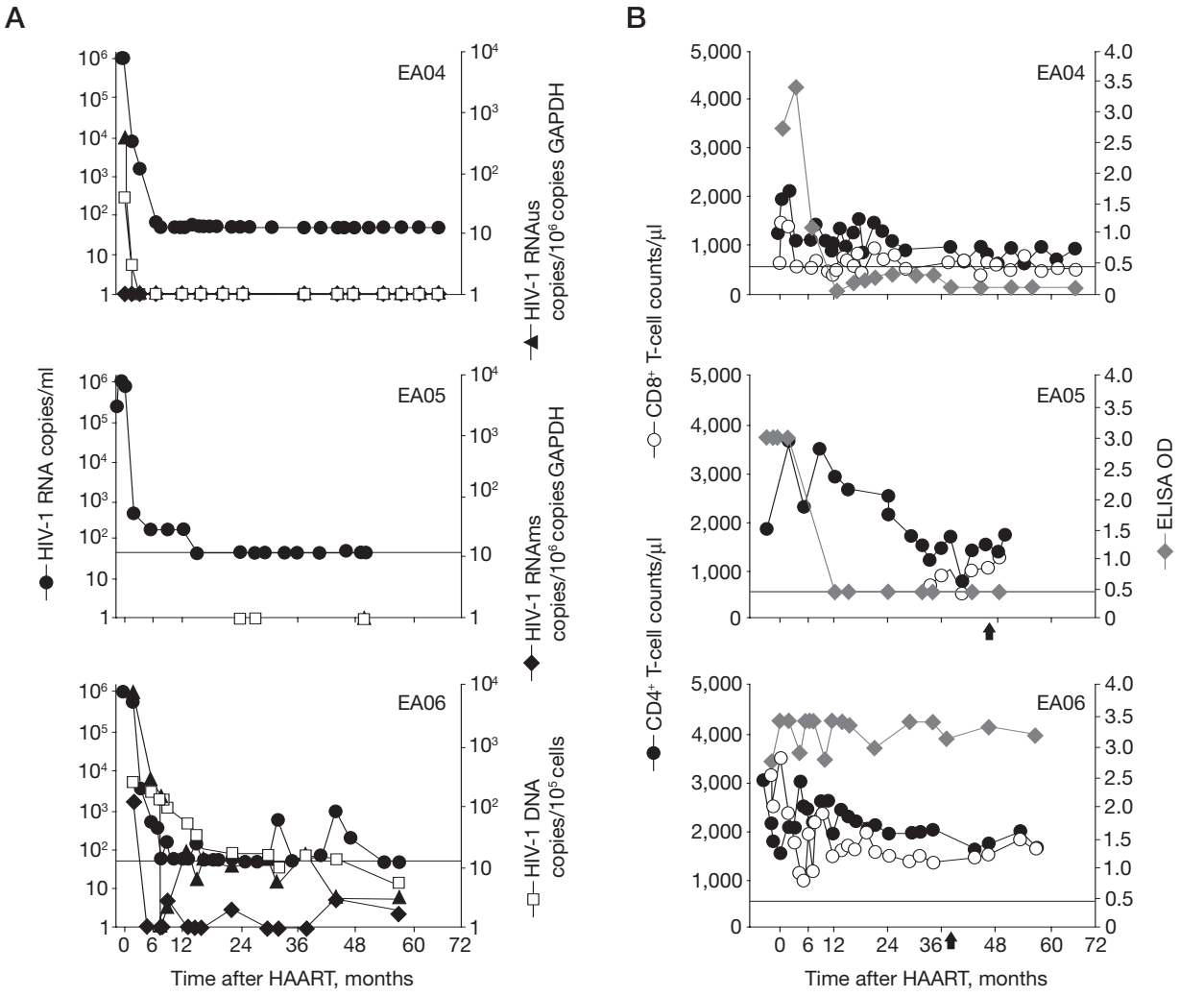
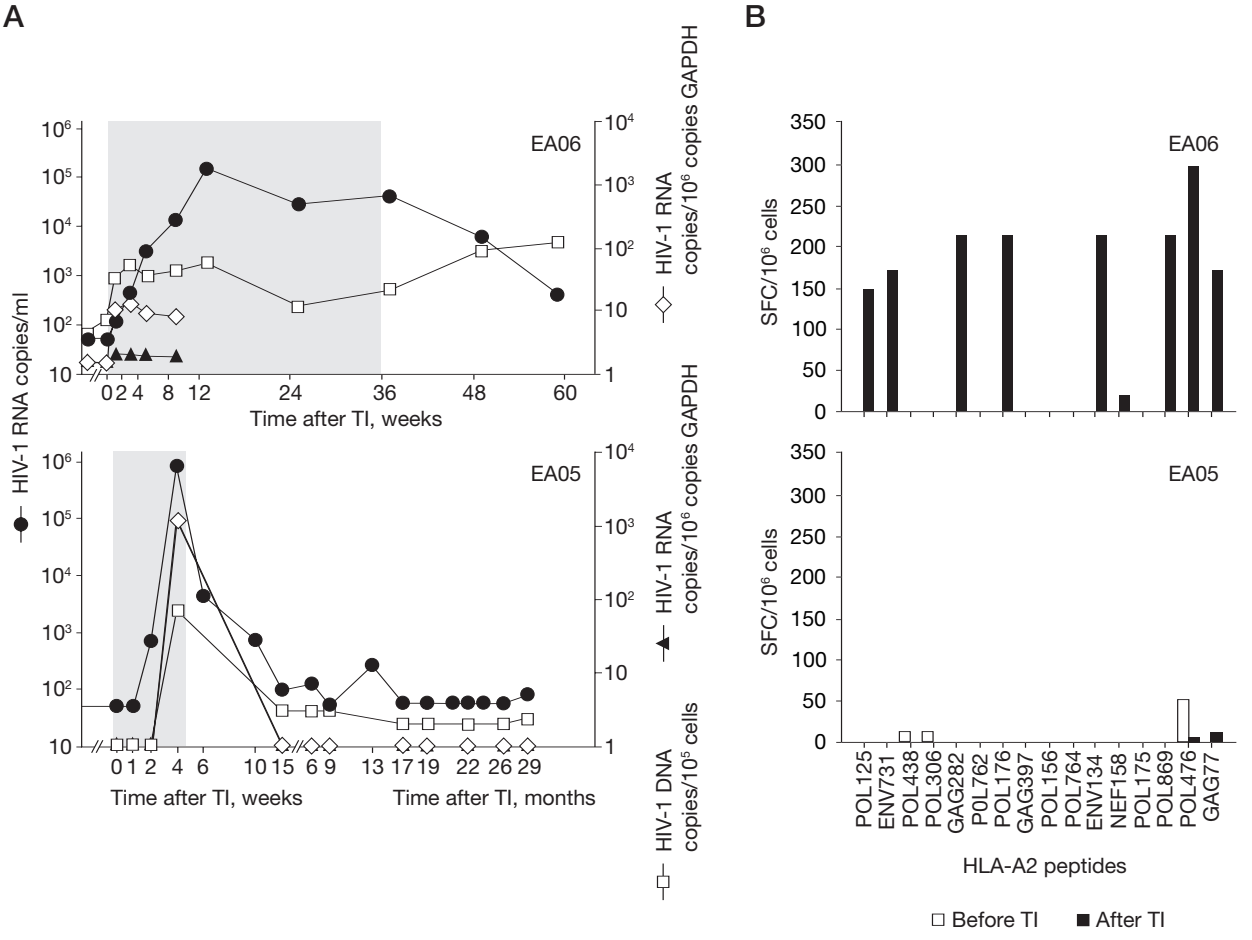


Table 3. HIV-1 peptide-specific CD8⁺ T-cell response in ART-treated children given as the total number of spot-forming cells (SFC)/10^6 cells

	HLA-A2 peptides															
	POL	ENV	POL	POL	GAG	POL	POL	POL	POL	POL	ENV1	NEF	POL	POL	POL	GAG
Patient	125	731	438	306	282	762	176	397	156	764	34	158	175	869	476	77
EA01 (49)*	–	–	–	–	–	–	215	–	–	–	–	–	–	–	–	–
EA02 (33)*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EA03 (34)*	150	–	135	–	–	–	–	–	–	–	–	–	–	–	–	–
EA05 (38)*	–	–	–	–	–	–	–	–	–	–	20	–	–	–	50	20
EA06 (37)*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*Time points (in months) when the EliSpot assay was performed.

Figure 2. Kinetics of viral replication during therapy interruption



(A) Change of plasma HIV-1 RNA, HIV-1 DNA, HIV-1 unspliced RNA (RNAus), and HIV-1 multiply spliced RNA (RNAms) parameters in one seropositive (EA06) and one seronegative (EA05) HIV-1-infected child. The shaded area indicates the period of therapy interruption. **(B)** HIV-1 peptide responses determined by EliSpot assay before (white columns) and after (black columns) therapy interruption. The results are expressed as spot-forming cells (SFC) per 10^6 cells. TI, therapy interruption.

viraemia preceded the viral rebound in PBMCs, possibly indicating that latently infected cells were in other reservoirs than the peripheral blood compartment [37]. Interestingly, a cellular HIV-1-specific immune response occurred in the child who remained for 36 months in therapy interruption, but did not occur in the child that had only short-term therapy interruption. This further supports the idea that the magnitude and duration of viral antigen exposure are crucial for the onset of immune response against HIV-1. In both cases, however, plasma viraemia was controlled after therapy re-introduction.

In conclusion, early ART is associated with long-term control of viral replication and good clinical and immunological outcomes. However, finding that HIV-1 persisted in seronegative aviraemic children strongly confirms that early ART is unable to eradicate infection, while it may greatly impair virus-specific immune responses. These findings are important for planning therapeutic strategies against HIV-1 in infants. Although the high grade of viral suppression suggests that simplified antiretroviral regimens may be sufficient to maintain long-term control of viral replication, the lack of immune response also suggests that therapy interruption in early treated infants might be preceeded by therapeutic vaccination.

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Disclosure statement

The authors have no conflict of interest.

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