HIV-1 virological remission lasting more than 12 years after interruption of early antiretroviral therapy in a perinatally infected teenager enrolled in the French ANRS EPF-CO10 paediatric cohort: a case report

Pierre Frange, Albert Faye, Véronique Avettand-Fenoel, Érianna Bellaton, Diane Descamps, Mathieu Angin, Annie David, Sophie Caillat-Zucman, Gilles Peytavin, Catherine Dollfus, Jérôme Le Chenadec, Josiiane Warszawski, Christine Rouzioux, Asier Sáez-Cirión, on behalf of the ANRS EPF-CO10 Pediatric Cohort and the ANRS EP47 VISCONTI study group

Summary

Background Durable HIV-1 remission after interruption of combined antiretroviral therapy (ART) has been reported in some adults who started treatment during primary infection; however, whether long-term remission in vertically infected children is possible was unknown. We report a case of a young adult perinatally infected with HIV-1 with viral remission despite long-term treatment interruption.

Methods The patient was identified in the ANRS EPF-CO10 paediatric cohort among 100 children infected with HIV perinatally who started ART before 6 months of age. Among them, 15 interrupted treatment while plasma HIV RNA was below 500 copies per mL and CD4 cell counts stable through to 18·6 years of age. After 11·5 years of control off therapy, HIV RNA was undetectable 1 month later. ART was discontinued by the family at some point between 5·8 and 6·8 years of age. HIV RNA load rebounded 27 months after treatment discontinuation.3 In 2013, an early combination ART (ART) is discontinued.1 In 2013, an infant infected with HIV-1 in utero seemed to have been functionally cured after rapid initiation of ART, but viral load rebounded 27 months after treatment discontinuation.1,3 Since 1996, 173 HIV-infected children have been included from birth in the French paediatric cohort ANRS EPF-CO10.4 100 of these children started combination ART before 6 months of age. Among them, 15 interrupted treatment while plasma HIV RNA was below 500 copies per mL and had at least one measurement recorded after treatment interruption. These children received ART for a median duration of 33 months. 13 children had viral rebound within 12 months of treatment interruption and one child had rebound after 3 years. One individual continues to have long-term virological remission. We report this case of ongoing virological and immunological control in a perinatally infected teenager (aged 18-6 years) who discontinued early ART more than 11-8 years ago.

Case report The female child was born in 1996 by spontaneous vaginal delivery at 37 weeks and 5 days of gestation to a woman with HIV-1 who had received zalcitabine monotherapy (instead of zidovudine monotherapy because of severe anaemia) from the 13th week of pregnancy. Maternal viral load at delivery was

www.thelancet.com/hiv Published online December 8, 2015  http://dx.doi.org/10.1016/S2352-3018(15)00232-5 1
42,000 copies per mL and the CD4 count was 81 cells per μL. Intrapartum zidovudine prophylaxis was not given. The newborn baby received a 6 week course of zidovudine, starting on the day of birth. Her plasma HIV RNA load was below 1000 copies per mL on day 3 of life (appendix p 5–7). HIV DNA was undetectable in her peripheral blood mononuclear cells (PBMCs) at 3 days and 2 weeks of age. PBMC-associated HIV DNA was detected for the first time at 1 month and plasma HIV RNA at 2 months of age (figure 1). ART with zidovudine, lamivudine, didanosine, and ritonavir was started at age 3 months, when the plasma HIV RNA load was 2170 000 copies per mL, and reduced the child’s viral load to below 500 copies per mL 1 month later. Between 4 months and 6-5 months of age, her nucleoside reverse transcriptase inhibitors were changed three times because of severe neutropenia. From 6-5 months, treatment consisted of stavudine, didanosine, and ritonavir. Plasma HIV RNA remained undetectable on ART, except for two transient rebounds at age 15 months and 21 months. Each time, interventions to improve treatment adherence re-established virological control, with undetectable viral load achieved 2 months later. Between 5-8 and 6-8 years of age, her family discontinued her medical follow-up and treatment. Because virus undetectable at 6-8 years of age, ART was not resumed. At the ages of 11-6 years and 13-9 years, low and transient viral blips were noted (510 copies per mL and 48 copies per mL, respectively). The patient has never exhibited symptoms of HIV infection, and her growth and neurological and pubertal development have been normal. Her CD4 cell percentage has remained stable. At the time of this report, at age 18-6 years, she had received no antiretroviral drugs for more than 11-8 years.

Between 2013 and 2015, detailed virological and immunological studies were done to characterise this case of long-term HIV-1 remission in a vertically infected patient (case A in this report).

Methods

Cases

In addition to case A, for purpose of comparison of immune responses, a viraemic perinatally infected adolescent (case B) and an adult natural HIV controller were also analysed. Case A was identified among patients followed up in the ANRS EPF-CO10 paediatric cohort as the only patient with long-term HIV remission after treatment interruption. Case B was an HIV-infected adolescent aged 14-6 years who was followed up in the ANRS EPF-CO10 paediatric cohort and who had uncontrolled viraemia after treatment discontinuation with a CD4 count of 537 cells per μL (20%) and an HIV RNA viral load of 1972 copies per mL at the time of the analysis. This patient had been treated with zidovudine, didanosine, and nelfinavir starting at 10 weeks of age for 3-5 years. At the time of the analysis, case B had been off ART for 11 years. We also assessed a treatment-naïve adult HIV controller from the ANRS CO21 CODEX cohort who had an HIV RNA viral load below 400 copies per mL of plasma for more than 5 years at the time of the analysis.

Research in context

Evidence before this study

We searched PubMed, without filters, for “HIV”, “cure”, “remission”, and “children” for papers published before September, 2015. One report in 2013 described the case of the so-called Mississippi child, a child infected with HIV-1 in utero who exhibited transient viral control after interrupting very early initiated combined antiretroviral therapy (ART). After this case was reported, several researchers suggested that tackling early HIV infection in children might provide an opportunity to obtain virological remission. However, viraemia rebounded in this child 27 months later, and other recent reports have described viral relapse after treatment interruption in perinatally infected children.

Added value of this study

In this study, we show that a perinatally HIV-infected young patient who started ART at 3 months of age has been able to control HIV viraemia to undetectable levels for over 11-8 years after treatment was discontinued by the family between 5-8 and 6-8 years of age. Two episodes of poor treatment compliance accompanied by high viral load showed that the patient was not able to naturally control viraemia without optimum ART early in life when viral load was high. Moreover, the patient’s major histocompatibility complex background is generally associated with rapid progression in the absence of treatment. HIV DNA is still at 18·6 years of life present in the cells from this patient and low levels of viral reactivation can be achieved in vitro.

Implications of all the available evidence

Our case provides, to our knowledge, the first evidence of durable virological remission in a perinatally infected young adult. The clinical history of this patient suggests a decisive role of the duration of ART to achieve a status that then allowed viral remission after treatment discontinuation. Although unusual, this case of virological remission in a perinatally HIV-infected patient shares some characteristics with adult patients with HIV who are in remission of infection; among them, the early, although not immediate, initiation of antiretroviral therapy. Together with the available evidence, this case supports the search for a possible window of opportunity early after HIV infection for ART implementation in children and adults that might lead to the establishment of immunovirological conditions that favour HIV control after treatment discontinuation.

See Online for appendix
Virological monitoring
In case A, HIV-1 antibody testing was done with western blot (BioRad New Lav Blot 1, Bio-Rad, Marnes-la-Coquette, France). Recent plasma samples (those taken from 16·7 years of age) were tested for HIV RNA, quantified with the Generic HIV-1 RNA Charge Virale kit (Biocentric, Bandol, France) using an ultrasensitive protocol (viral particles were concentrated by high speed centrifugation of high plasma volume) and the Cobas AmpliPrep/COBAS TaqMan HIV-1 kit version 2.0 (Roche, Paris, France). PBMC-associated HIV DNA was measured with the Generic HIV DNA cell kit (Biocentric) with an ultrasensitive protocol (by testing six replicates). The virus was subtyped by phylogenetic analysis of the reverse transcriptase gene sequence.

Pharmacological testing
In case A, at age 18·3 years, to detect non-prescribed cART we tested for at least 15 different commercially available antiretroviral drugs in the plasma by liquid chromatography coupled with tandem mass spectrometry (Acquity UPLC/TQD, Waters, Milford, MA, USA) with a modified method. For intracellular cytokine staining, PBMCs were incubated for 6 h with overlapping peptide pools encompassing HIV-1 clade B gag, pol, and nef (2 μg/mL; NIH AIDS Reagent Program). Anti-CD28–anti-CD49d co-stimulation (1 μL/mL) and the anti-CD107α marker were used with each stimulation condition. Phorbol myristate acetate (80 ng/mL) and ionomycin (1 μg/mL; Sigma-Aldrich, St Quentin Fallavier, France) were used as a positive control. Golgi stop (1 μg/mL; BD Biosciences, Le Pont de Claix, France) and Brefeldin A (10 μg/mL; Sigma-Aldrich) were added 30 min after the start of all incubations. Cells were then stained with the LIVE/DEAD Fixable Aqua Dead cell Stain kit (Life Technologies, Saint Aubin, France) and anti-CD3 and anti-CD8 antibodies. The Cytofix/Cytoperm kit (BD Biosciences) was used for intracellular staining of antibodies against interferon γ, interleukin 2, and tumour necrosis factor α.

Detection and amplification of HIV-1 from peripheral blood CD4 cells
1 million CD4 cells were activated with phytohaemagglutinin (1 μg/mL) and interleukin 2 (100 IU per mL). 2 days later, the cells were washed, mixed with 3×10⁶ pooled phytohaemagglutinin-activated CD4 cells from two healthy donors and cultured for 22 days with interleukin 2. Viral replication was monitored in the culture supernatant with the Generic HIV-1 RNA Charge Virale test and by p24 ELISA (ZeptoMetrix, Buffalo, NY, USA).

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Figure 1: Monitoring of HIV-1 viral load and CD4 cell counts in the child
HIV-1 RNA viral load (circles) and CD4 cell percentages (triangles) during the follow-up period, from birth to age 18·6 years. Detectable HIV-1 RNA counts (red circles) and the assay detection limit in case of undetectable viral load (blue circles) are shown. Shaded areas are the periods during which the child received antiretroviral drugs. 3TC=lamivudine. D4T=stavudine. DDI=didanosine. RTV=ritonavir. ZDV=zidovudine.
Results

At age 3 days, the western blot showed reactivity to all (gp160, gp110, p68, p55, gp41, p40, p34, and p24) but one (p18) HIV-1 proteins. At age 17 years, western blot showed strong reactivity to p68, p55, p40, p34, and p24, weaker reactivity to gp160 and gp41, and no reactivity to gp110 or p18. HIV RNA remained undetectable at ages 16·9 years, 18·3 years, and 18·6 years (all <20 copies per mL by the Roche assay and <9, <4, and <7 copies per mL by the Biocentric assay, respectively). PBMC-associated HIV DNA counts were stable at ages 16·7 years, 16·9 years, 18·3 years, and 18·6 years (2·1 log10 copies per 1 million PBMCs at age 16·7 years, 2·2 log10 copies at 16·9 and 18·3 years, and 2·5 log10 copies at 18·6 years; appendix p 7). These values are within the range reported in adult HIV controllers after treatment in the ANRS VISCONTI study1 and among the lowest reported in untreated perinatally infected children.10 The virus was HIV-1 subtype H.

At age 18·3 years, plasma concentrations of all antiretroviral drugs, measured concomitantly with plasma HIV RNA, were below the respective detection limits, confirming the absence of recent ART use.

The HLA genotype showed homozgyosity at several loci: A*2301-, B*1503/4101, C*0210/0802, DRB1*1101-, and DQB1*0602-. T-cell activation marker expression was normal at age 18·3 years (15% of T cells were CD3+/HLA-DR+, 21% were CD8+/HLA-DR+, and 12% were CD4+/HLA-DR+).15 CD8 T cells produced all cytokines tested and degranulated in response to phorbol myristate acetate and ionomycin stimulation (figure 2). However, responses to pools of HIV-1 peptides were barely detectable (figure 2 and appendix p 2). The HIV-specific response was weaker in the study patient (case A) than in a viraemic perinatally infected adolescent (case B) and an adult HIV controller who were assessed during the same period (0·069% of IFN-γ+ CD8 T cells from case A after stimulation with gag, pol, and nef pools vs 1·295% for case B and 1·079% for the HIV controller; figure 2 and appendix p 3). The patient’s CD8 T cells showed no capacity to suppress HIV-1 infection of CD4 T cells (0·23 log p24 decrease CD4 vs CD4:CD8; 0·77 for

Figure 2: HIV-specific T-cell responses after more than 10 years of remission

Frequencies of CD8 T cells (A) and CD8-negative T cells (B) producing IFN-γ in response to phorbol myristate acetate and ionomycin stimulation and to pools of gag, pol, and nef peptides. The test was done simultaneously in the patient (case A), a viraemic perinatally infected adolescent (case B), and an adult HIV controller. (B and E) Relative capacity of CD8 T cells and CD8-negative cells from the same three patients concomitantly to exert (in response to a pool of gag peptides) one, two, three, or four functions (to produce interferon γ, interleukin 2, and tumour necrosis factor α and degranulation marker CD107α expression, or or multiple simultaneous combinations). The size of the circles is proportional to the cumulative total response. (C) Concentrations of HIV-1 p24 detected in culture supernatants after superinfection with HIV-1 BaL of CD4 T cells from the HIV controller, case A, and case B, cultured with or without autologous CD8 T cells (CD4:CD8 ratio 1:1). Bars show means and SDs. (F) Concentrations of autologous HIV-1 RNA (maximum 7810 copies per mL, in red) and p24 (maximum 122 pg per mL, in purple) in culture supernatants after stimulation of CD4 T cells from case A with phytohaemagglutinin. Heterologous feeder CD4 T cells were added to the culture on day 7. p24 production after activation of CD4 T cells from case B patient are shown as a reference (green triangles). IFN-γ=interferon γ. PMA=phorbol myristate acetate.
case B and 2·68 for the HIV controller; figure 2). By contrast, her HIV-specific CD4 T-cell response, as estimated in the CD8-negative fraction, was within the same range as in case B and the HIV controller (0·220% of IFN-γ+ CD4 T cells from case A after stimulation with gag, pol, and nef pools vs 0·128% for case B and 0·885% for the HIV controller; figure 2 and appendix p 3). Her HIV-specific T cells did not show superior poly-functionality (figure 2 and appendix p 4).

After stimulation of CD4 cells, HIV-1 RNA and p24 were detected in the culture supernatant at all timepoints (figure 2). Her CD4 cells were susceptible to infection with HIV-1 BaL in vitro (figure 2).

**Discussion**

We report the case of a perinatally infected teenager who experienced long-term virological remission from HIV-1 infection lasting for more than 12 years after cessation of early ART. Because HIV DNA and RNA were undetectable in her blood at age 3 days, the patient was probably infected very late in gestation or during delivery. We cannot exclude that the zidovudine-based postnatal prophylaxis could have contributed to the late positive HIV-1 results on PCR in the baby. Indeed, in a recent analysis of the performance of DNA and RNA tests for HIV-1 results on PCR in the baby. Indeed, in a recent analysis of the performance of DNA and RNA tests for early diagnosis of perinatal HIV-1 infection in 1567 French infants, 11% of infected children had negative PCR results at 1 month during antiretroviral prophylaxis. At age 3 months, her plasma HIV RNA load was high and her CD4+ T-cell percentage decreased. These findings suggest that she was infected by a highly replicative and pathogenic virus. Although ART rapidly controlled viraemia and normalised the CD4+ T-cell percentage, she experienced two viral rebounds, apparently associated with poor adherence, during her second year of life. These episodes showed that the patient was unable to control the virus without treatment at this age and suggest that her long-term treatment contributed to the creation of an optimum situation in which she could achieve durable remission.

The HIV-1 subtype that infected our patient (H) is rare and almost exclusively found in central Africa. However, this clade did not seem to contribute to the control of HIV replication after treatment in our patient for two reasons. First, we identified, in our previous reports, similar immunovirological features in children infected with strains clustering with subtype H to those in patients harbouring more common HIV-1 subtypes. Second, the severe immune deficiency noted in our patient’s mother at the time of delivery and the high HIV-1 viraemia measured in both mother and baby before the start of ART suggested that the transmitted strain was not less pathogenic than viruses belonging to more common subtypes.

Several characteristics of this patient resemble those of adult HIV-1 controllers after treatment who were enrolled in the ANRS VISCONTI study, but differ from those of adult HIV controllers. First, we found homozygosity at several HLA loci, which has previously been linked to rapid disease progression. Generally unfavourable HLA genotypes also commonly occur in adult post-treatment controllers. Second, our patient showed weak T-cell activation and HIV-specific CD8 T-cell responses. The use of HIV-1 subtype B consensus peptides can underestimate the response in subtype H infection, but also the intensity of the patient’s HIV-specific CD4 T-cell responses was close to that of the two control patients we analysed. Overall, our results suggest that the CD8 T-cell response is not the major force keeping control of viraemia in this patient. The precise mechanisms associated with HIV control after treatment remain unclear. Recent data obtained in adult patients from the ANRS VISCONTI study suggest that these patients carry natural killer cells with a particular phenotype and improved capacity to inhibit HIV infection. Whether the innate immune response plays a part in the control of infection in the case of this patient remains to be analysed. Another possibility is that early initiated and prolonged ART favoured the archiving of less fitted or less inducible viruses. The patient still harbours infected cells from which HIV replication can be induced in vitro, albeit with difficulty. Weak contribution of central memory T cells to the HIV-1 reservoir has been reported in adult HIV-1 controllers after treatment. Although we did not address this issue in our patient, neonates have lower frequencies of central-memory-phenotype CD4 cells than adults, which might protect these cell populations from infection during childhood. Moreover, in children who are treated early, transitional memory CD4 cells make a larger contribution to the viral reservoir than do longer-lasting memory CD4 cells. However, no cases of long-term post-treatment remission have been previously reported in children, even in those with very low HIV blood cell reservoir concentrations before ART cessation. Because indefinite ART is recommended for children, only a very small proportion of early treated, virologically suppressed children have treatment interruption, which could explain the reported rarity of childhood post-treatment controllers worldwide. Moreover, viral suppression on ART is more difficult to achieve and sustain in children than in adults, and therefore we cannot exclude the possibility that a lesser proportion of early treated children achieve remission because of poor viral control during treatment.

The so-called Mississippi child maintained undetectable viraemia for 27·6 months after ART discontinuation. Although ART was also started very early, there are noteworthy differences with our patient. First, the Mississippi child’s plasma HIV RNA load was 19182 copies per mL 31 h after birth, and she therefore might have been infected earlier during pregnancy than our patient. Second, our patient was exposed longer to viral antigens after delivery, because the Mississippi child started ART immediately and had detectable viraemia for less than 1 month. This early treatment limited the size of the viral reservoir in the Mississippi child, whose HIV
DNA titres were lower than in our patient despite shorter treatment. Whether longer exposure to viral antigens might be important for achieving remission after treatment is unknown. Moreover, because the immune system is immature in young children, longer ART might be needed to achieve an optimum balance between a small pool of infected cells and efficient immune responses. Also unknown is whether the brief exposure to the virus during the two short episodes of viral relapse during the second year of life of the patient contributed to the optimum maturation of the immune response against HIV. Finally, durable HIV-1 remission is likely to be associated with intrinsic host and viral factors favoured by early and lengthy ART.

This case shows that early and prolonged ART can be followed by off-therapy viral control lasting several years in a perinatally infected child with no known features associated with natural control, as observed in HIV controllers who never receive antiretroviral treatment. A better understanding of the underlying mechanisms might lead to therapeutic approaches that enable more perinatally infected patients to discontinue treatment and achieve HIV-1 remission for long periods.

Contributors
PF, AF, EB, CD, JW, CR and AS-C participated in the conception and design of the study. VA-F, EB, DD, MA, AD, SC-Z, GP, and JLC did experiments or collected and analysed data, or both. All authors interpreted data. PF and AS-C drafted the manuscript including figures and tables; all authors revised it critically for important intellectual content and have approved the final version submitted for publication.

Declaration of interests
PF reports consultancy honoraria from MSD, Bristol-Myers Squibb and Janssen Cilag; travel support from MSD, Gilead Sciences, Bristol-Myers Squibb, Janssen Cilag, and ViVi Healthcare. VA-F reports consultancy honoraria from ViVi, and travel support from MSD. DD reports consultancy honoraria from ViVi Healthcare, Gilead Sciences, Janssen Cilag, MSD, and Bristol-Myers Squibb; travel support from ViVi Healthcare and Gilead Sciences. GP has received grants and consultancy honoraria from Bristol-Myers Squibb, Gilead Sciences, ViVi Healthcare, Janssen and Merck. JW reports grants from ViVi Healthcare, Parexel, Abbvie. AS-C reports grants from MSD, consultancy honoraria from MSD, ViVi healthcare, Gilead, and Bristol-Myers Squibb. All support for all authors outside the submitted work. All other authors declare no competing interests.

References