

CASE REPORT

Undetectable Plasma HIV-1 RNA with Strong gag-pol Specific Interferon- γ ELISPOT Response in an HIV-1 Clade A/E-Infected Child Untreated with Antiretroviral Therapy

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In children older than 18 months of age, HIV diagnosis is based on confirmed positive HIV serology;^{1, 2} however, in children younger than 18 months, DNA and RNA PCR are necessary because of persistent maternal HIV antibody. In the past 5 years, plasma HIV-1 RNA measurement or viral load has been an important tool for disease and treatment monitoring. This is usually detected at high levels in children untreated with antiretroviral therapy (ARV). Our 7-year old patient fits the diagnostic criteria for HIV infection. Nevertheless, her plasma HIV-1 RNA and DNA PCR, which were performed by tests validated for A/E clade were negative in the absence of ARV.

SUMMARY We report a 7-year HIV-1 clade A/E-infected child untreated with antiretroviral therapy who had positive HIV antibody testing but undetectable plasma HIV-1 RNA by Roche Amplicor version 1.5 and bDNA version 3.0. DNA PCR was positive by methods using gag/pol primers but not env/pol primers. The patient had strong HIV-1-specific cytotoxic T lymphocyte responses, which likely contributed to her low viral burden and undetectable plasma HIV-1 RNA.

CASE REPORT

A 7-year old ARV-naïve girl had been enrolled in HIV-NAT 010 trial; an immediate versus deferred ARV initiation trial, since December 2002 and was randomized to the deferred arm. At study week 0, HIV RNA PCR was undetectable (below 50 copies/ml) by Roche Amplicor version 1.5 (Roche diagnostic systems, Inc., Branchburg, NJ, USA).

She was diagnosed with HIV by enzyme immunoassay (EIA) in November 2002 at a local hospital because of HIV infection was detected in her parents. Repeated HIV EIA of her serum samples at time of screening into HIVNAT 010 was strongly positive. Her clinical staging

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was clinical class A by Center of Disease Control and Prevention (CDC) criteria because of lymphadenopathy and frequent upper respiratory tract infections. The percentage of her CD4 cell count was in the CDC immune category 2 (moderate immunosuppression).

Her parents and 5-year old brother are HIV-positive. Her brother who is also enrolled in HIVNAT 010 has positive HIV EIA and detectable viral load in the absence of ARV.

Physical examination revealed a child with normal weight, height and development. She had bilateral lymphadenopathy at neck; otherwise her physical examination was normal.

Further investigations in this

child are shown in Table 1. HIV antibody performed by a different method, particle agglutination, showed strongly positive results on 2 separate occasions. Repeated plasma HIV-1 RNA RT PCR by Roche Amplicor version 1.5 was undetectable (< 50 copies/ml). Even though the patient fulfilled the diagnostic criteria for HIV infection according to the Thai guidelines.¹ In the light of the unusual finding of undetectable viral load, we were concerned that she might have a false positive antibody result. Therefore, we performed HIV-1 DNA PCR, which was negative. This test used env/pol primers and had been validated for HIV A/E clade (Tirawatnpong T: personal communication). This test is offered as routine testing at Chulalongkorn University Hospital. We tested 10 HIV-negative and 10 HIV-positive

samples using this method in our laboratory and found 100% sensitivity and specificity. A repeat HIV DNA PCR with this same method was negative.

In an attempt to document her HIV status, a month later, we performed western blot (HIV Blot 2.2 Western Blot Assay, Genelabs Diagnostics, Singapore), plasma HIV-1 RNA quantification by bDNA, HIV-1 DNA PCR using different primers (gag/pol) which was sent to two different laboratories,^{3,4} cytotoxic T lymphocyte (CTL) assay using vaccinia-induced interferon-gamma (IFN- γ) ELISPOT⁵ and standard HIV culture.⁶ Results are shown in Table 1. Again, her plasma HIV-1 RNA quantification was undetectable at the 50 copies/ml cut-off by the bDNA method. Her HIV culture was negative at

Table 1 HIV serologic and virologic testing results

Date	Serologic testing		Virologic testing		Others
	Tests	Results	Tests	Results	
December 2002	HIV ELISA	Positive (3+)	Plasma HIV-1 RNA-Amplicor	< 50 copies/ml	CD4+* 20% (606 cells/mm ³) CD4/8 ratio 0.49
January 2003	HIV ELISA	Positive (3+)	Plasma HIV-1 RNA-Amplicor	< 50 copies/ml	-
February 2003	HIV Ab -PA	Positive	HIV DNA PCR (env/pol primers)	Negative	-
March 2003	HIV Ab - PA	Positive	HIV DNA PCR (env/pol primers)	Negative	CD4+ 20% (991 cells/mm ³)
April 2003	Western blot	Positive	Plasma HIV-1 RNA-bDNA	< 50 copies/ml	CTL-strongly positive for gag/pol
			Two HIV DNA PCR methods(gag/pol primers)	Positive	-
			HIV culture	Negative at day 28	-
May 2003			Plasma HIV-1 RNA-Amplicor	< 50 copies/ml	CD4+ 23% (816 cells/mm ³)

Ab: antibody, PA: particle agglutination, RNA: ribonucleic acid, DNA: deoxyribonucleic acid, PCR: polymerase chain reaction, bDNA: Bayer branched-DNA assay version 3.0, Amplicor: Roche Amplicor version 1.5, CTL: Cytotoxic T lymphocytes assessed by interferon-gamma ELISPOT

*Age-adjusted normal value for this patient is CD4 \geq 25% or \geq 500 cells/mm³

day 28. However, both her western blot and DNA PCR from the two laboratories were positive. Viral sequencing revealed HIV-1 A/E clade. She had strong CTL responses to gag and pol (Fig. 1). At her most recent follow up in January 2004, the plasma HIV-1 RNA remained below detection.

DISCUSSION

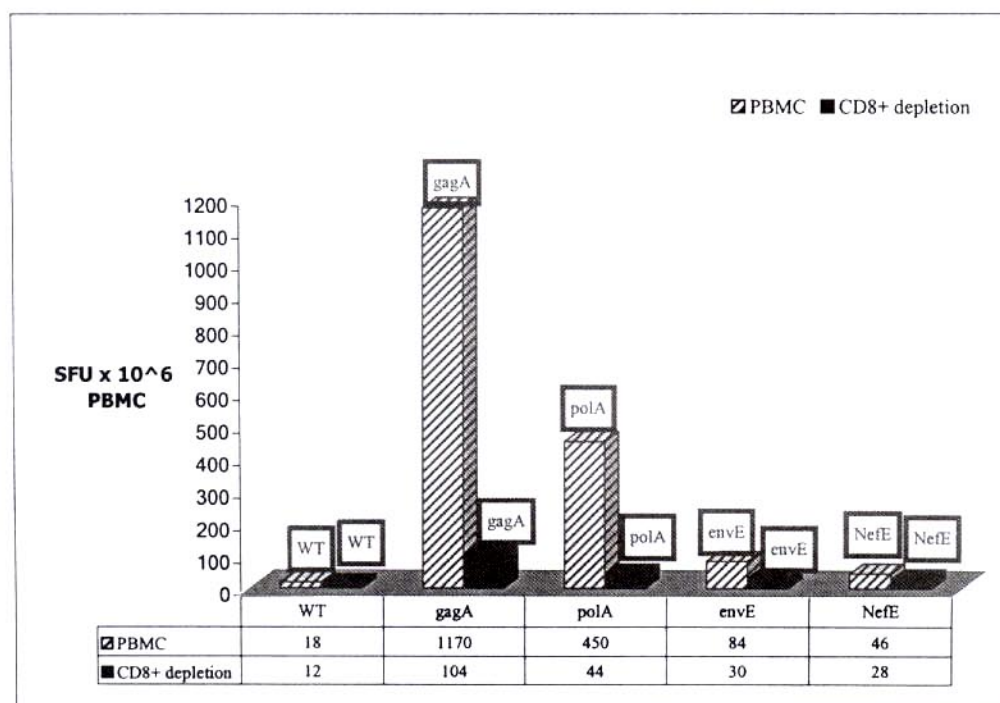
Our patient's diagnosis was in question when HIV antibody tests by EIA and particle agglutination were strongly positive but plasma HIV-1 RNA by both Roche Amplicor version 1.5 and Bayer bDNA version 3.0 methods was undetectable in the absence of ARV. In addition, DNA PCR by a validated in-house method using env/pol

primers was also negative. Further testing confirmed her HIV diagnosis with positive western blot and HIV DNA PCR using gag/pol primers. A strong CTL response to gag/pol also supported the diagnosis. She is currently healthy and not on ARV. According to HIV-NAT 010 trial, she will start ARV only if CD4 is < 15% or she experiences clinical progression.

In general, DNA and RNA PCR have high positive and negative predictive values, and specificity and sensitivity for HIV detection.⁷⁻⁹ However, most tests are validated for use in HIV B clade, the most common clade in the Western hemisphere. It is not surprising that most reports of false negative DNA PCR have been for cases when it is

used in non-B HIV clade. Kline *et al.*¹⁰ reported a Sudanese infant with HIV clade C who had negative HIV DNA PCR. This child had detectable HIV RNA PCR by Roche Amplicor version 1.0 on two occasions with a mean viral load of 25,632 copies/ml.¹⁰ An adult who acquired HIV in Thailand where clade A/E predominates had extremely high plasma HIV-1 RNA in the presence of negative HIV DNA PCR.¹¹ Both the DNA PCR tests used in our patient had been validated for A/E clade.

It is uncommon to have undetected RNA PCR in the absence of antiretroviral treatment. Reports of false negative HIV-1 RNA RT PCR have been attributed to the use of Roche Amplicor version 1.0 in non-



Patient's peripheral blood mononuclear cells (PBMC) and control cells (patient's PBMC depleted of CD8+ T cells) were stimulated by recombinant Vaccinia HIV-1 viral peptide constructs and assessed for cytotoxic T lymphocyte responses (expressed by interferon-gamma release from cells or spot forming unit, SFU). Gag and pol elicited the most prominent responses.

Fig. 1 Recombinant Vaccinia Interferon-gamma ELISPOT

clade B, which could be overcome by using bDNA method or Roche Amplicor version 1.5.^{10,12-14} An African woman with clade G HIV had high viral load by bDNA (460,000 copies/ml) but undetected viral load by both the Amplicor Monitor and NASBA assays.¹⁵

Our patient has the common A/E HIV clade found in Thailand for which the Roche Amplicor version 1.5 and bDNA version 3.0 used have been validated. Therefore, we believe that the undetectable plasma HIV-1 RNA in our patient is not explained by the A/E clade, but rather by low-target HIV copy number and the variation in the selected primers. Brisk CTL response such as the one seen in our patient has been reported in persons with low viremia despite no antiretroviral treatment. These individuals tend to have no or slow disease progression and are considered long-term non-progressors (LTNP).¹⁶⁻¹⁸ Our patient has not experienced serious clinical events and has maintained her CD4 above 15%, the level under which there exists significant risk of opportunistic infections.

The discrepancy in results between the two DNA PCR methods is likely explained by the use of different primers. Env/pol primer-based DNA PCR was negative, while the gag/pol-based test was positive. Her low CTL response to env may be due to env sequence variation or imperfect binding of HLA-env antigen rendering it less immunogenic. The currently negative HIV culture at day 28 does not disprove her HIV diagnosis, as standard HIV culture can be negative in some cases and may require longer culture time.⁶ For LTNPs,

high input HIV culture with CD8+ T cell depletion may improve the value of viral isolation.

In conclusion, our patient represents an unusual case of an ARV-naïve child with negative plasma HIV-1 RNA by Roche Amplicor version 1.5 and Bayer bDNA version 3.0, and negative DNA PCR using env/pol primers in the presence of strongly positive HIV antibody and IFN- γ ELISPOT-based CTL responses. The undetectable plasma HIV-1 RNA is likely due to low viremia. This child may represent a long-term non-progressor.

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