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Identification of human immunodeficiency virus-1 specific CD8⁺ and CD4⁺ T cell responses in perinatally infected infants and their mothers

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Abstract

Background—There are few data describing the specificity, breadth and magnitude of T cell responses to HIV-1 in infancy.

Methods—HIV-specific CD8⁺ and CD4⁺ T cell responses to peptide pools representing Gag, Env, Pol, Nef and the regulatory regions (Reg) were simultaneously measured in 18 perinatally infected infants and 14 of their chronically infected mothers, using a whole blood interleukin-2 and interferon- γ flow cytometric intracellular cytokine staining (ICS) assay.

Results—HIV-specific CD8⁺ T cell responses were detected in all the infants aged 6 weeks and older (range 0.1–6.62%) and their mothers (range 0.1–4.89%). HIV-specific CD4⁺ T cell responses were detected in 33% of the infants (range 0.11–0.54%) and 73% of the mothers (range 0.16–0.84). CD8⁺ T cell responses in the mothers were almost equally spread between the variable (Nef, Reg and Env) and conserved proteins (Gag and Pol). Conversely, CD8⁺ T cell responses to the more variable proteins dominated in the perinatally infected infants comprising 74% of the total response. Interestingly, mothers and infants shared responses to at least one peptide pool, whereas only one mother–infant pair shared a peptide pool targeted by CD4⁺ T cells. Two in-utero–infected infants tested at birth had CD8⁺ T cell responses, and one of them had an Env-specific CD4⁺ T cell response.

Conclusion—Our observations that HIV-specific CD8⁺ and CD4⁺ T cell responses can be detected in perinatally infected infants from 6 weeks of age and that CD8⁺ T cell responses predominantly target the variable proteins have important implications for HIV vaccine design.

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Keywords

HIV-specific T cell responses; HIV-infected infants and mothers

Introduction

Mother-to-child transmission of HIV-1 remains a major public health emergency with an estimated 30% of pregnant women attending public antenatal clinics in South Africa seropositive for HIV-1 (Department of Health South Africa, 2006). In the absence of antiretroviral therapy, about 20% of infected mothers will transmit the virus during pregnancy (*in utero*) or during delivery (intrapartum) and the prognosis of infected children is poor [1]. Although programs to reduce transmission and ameliorate the course of disease of infected children are being implemented globally, incomplete coverage of these programs keeps perinatal HIV infection at the forefront of threats to the survival and health of children in sub-Saharan Africa.

Perinatally infected infants usually have a more rapid disease progression than do HIV-1-infected adults [2-4]. Infants generally have extremely high viral loads that decrease to the viral set point more slowly than in adults. Reasons for this presumably include an immature immune system less able to control the virus, together with higher thymic output and increased numbers of circulating CD4⁺ T cells serving as targets for the virus. In addition, because the mother and infant share at least three of the six HLA class I alleles, viral variants that have already evaded the maternal cytotoxic T lymphocyte (CTL) response may be transmitted, thus compromising the infant's ability to mount an effective CTL immune response [5-8].

The first few months of life of perinatally infected infants is equivalent in terms of the duration of infection to the immediate few months following acute infection in adults. Although CTLs appear early in acute infection in adults, original studies in infants found that these responses were weaker and emerged later than in adults [9-13] and have only been found infrequently in children who are less than 6 months old [12,14]. However, these studies were undertaken using techniques that were not as sensitive as those that are presently available.

Many studies have characterized HIV-specific T cell responses in adults, but to date only one study has been conducted concerning the specificity, breadth and magnitude of T cell responses in infancy [15,16], and no study has compared these parameters in both mothers and their matched infants. To address this gap, the aim of this study was to simultaneously measure HIV-specific CD8⁺ and CD4⁺ T cell responses to HIV-1 peptide pools to Gag, Env, Pol, Nef and the regulatory regions (Reg) in perinatally infected infants and to compare infants' responses with those of their mothers, using a whole blood intracellular cytokine staining (ICS) assay. The whole blood assay was chosen as it uses only small volumes of blood, making it possible to study infants. Other studies have tended to rely only on interferon- γ (IFN- γ) to detect CD8⁺ T cell responses; however, as immune responses are complex, it has been shown that the use of one cytokine alone results in reduced sensitivity of the assay [17]. As our interests were whether there was an HIV-1-specific T cell response or not, rather than which cytokines were produced by which cells, and in order to maximize detection of responses for both CD8⁺ and CD4⁺ T cells, a combination of IL-2 and IFN- γ detection was used.

Participants and methods

Study participants

A total of 18 HIV-1-infected children and their mothers were included in this study (Table 1 Table 1). Of these, 12 infants were recruited from Chris Hani Baragwanath Hospital in Soweto and six infants were recruited from Coronation Hospital in Johannesburg, South Africa. As previously described, HIV-infected women and their newborns were recruited postpartum with informed consent into a protocol (approved by the investigators' institutional review boards) that prospectively followed the mother–infant pairs with regular blood samples [18]. We classified six of the infants as in-utero infections, as they were PCR positive at birth and six as intrapartum infections, as they were PCR negative at birth and PCR positive at 6 weeks. Six infants had no birth sample and therefore the timing of transmission could not be determined. None of the 18 infants in this study were breastfed. Blood for the ICS assays described here were collected from three of the infants at birth only, from one infant at birth and 6 weeks and from 14 infants at between 6 and 10 weeks of age. Blood for the ICS assay among mothers were collected at the same time points as among infants. Thirteen of the mothers were given single dose nevirapine to prevent mother-to-child transmission of HIV-1. Only one woman received an effective regimen with nevirapine, lamivudine and stavudine. We were not able to obtain samples for ICS from four of the mothers.

HIV-1 quantitation

HIV-1 RNA levels (expressed as log₁₀ units) were quantitated using the Roche Amplicor RNA Monitor assay version 1.5 (Roche Diagnostic Systems Inc., New Jersey, USA) with a lower detection limit of 400 HIV-1 RNA copies/ml. Samples with viral loads above 750 000 RNA copies/ml were diluted to determine the absolute value.

HIV-1 peptides

The peptides for the study were provided by the Immunology Laboratory at AIDS Virus Research Unit, NICD. These comprised a total of 396 overlapping peptides spanning nine HIV-1 subtype C gene regions (Gag, Pol, Nef, Env, Tat, Rev, Vif, Vpu and Vpr). The peptides were synthesized as 15–18 mers overlapping by 10 amino acids, with the exception of Nef, which overlapped by 11 amino acids. Gag, Vif, Vpu and Vpr were designed to match consensus C, whereas gene regions that had been selected for a HIV-1 subtype C vaccine (Du151 and Du179) [19] were used to design Pol, Nef, Tat, Rev and gp160 peptides. Peptides were pooled, with pools containing the following numbers of peptides: Gag, 66; Pol, 92 (without integrase); Env, 114; Nef, 50; and the regulatory region (Tat, Rev, Vif, Vpu and Vpr), 70. Peptides were diluted to at an initial concentration of 10 mg/ml in 100% dimethyl sulphoxide (DMSO) and were pooled in phosphate-buffered saline (PBS), in which the final DMSO concentration was less than 0.5% at 40 µg/ml/peptide stock.

Intracellular cytokine staining

Blood was collected in sodium heparin tubes and stimulation performed within 6 h of sample collection. Briefly, 200 µl whole blood was stimulated with a final concentration of 10 µg/ml of peptide together with 1 µg of each of the costimulatory antibodies CD28 and CD49d (BD Biosciences, San Jose, California, USA) in the presence of the transport inhibitor Brefeldin A (10 µg/ml; Sigma-Aldrich, St. Louis, Missouri, USA). To monitor spontaneous cytokine release, the equivalent amount of DMSO as in the peptide tube together with the costimulatory antibodies was prepared for each patient. Staphylococcus enterotoxin B (SEB) was used as a positive control. Samples were incubated for 6 h at 37°C, after which they were cooled to 18°C overnight. Twenty microliters of EDTA was added for

15 min, following which samples were transferred to fluorescence activated cell sorting (FACS) tubes where the red blood cells were lysed for 10 min at room temperature (RT) using 2 ml FACS lysing solution (BD Biosciences). Samples were centrifuged and then permeabilized using 500 μ l FACS permeabilizing solution 2 (BD Biosciences) for 15 min at RT. Permeabilized cells were washed twice and stained with CD3 allophycocyanin (APC), CD8 peridinin chlorophyll (PerCP) and IFN- γ phycoerythrin and IL-2 phycoerythrin (BD Biosciences) for 60 min at RT in the dark. Following the last wash, cells were resuspended in 150 μ l of 1% paraformaldehyde (1 : 10 in PBS) and placed at 4°C until acquisition within 24 h. Where possible, 70 000 CD3⁺ cells were acquired per sample using a FACS calibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California, USA). Analysis was performed using FlowJo Software (Tree Star, San Carlos, California, USA), CD8⁺ T cells were identified as the CD3⁺CD8⁺ cells and CD4⁺ T cells were defined as the CD3⁺CD8⁻ within the lymphocyte gate. A response was defined as positive if it was 0.1% or more after subtraction of the background.

Statistical analysis

Mann–Whitney *U*-test, Wilcoxon signed-rank test, Spearman correlation coefficient and Fisher exact test were performed using SPSS version 15.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Study population

The median viral load at 6–10 weeks among infants and their mothers were 5.89 log copies/ml (4.3–6.46 log) and 4.22 log copies/ml (2.6–5.67 log), respectively.

CD8⁺ but not CD4⁺ T cell responses are common in 6–10-week-old infants

HIV-specific CD8⁺ T cell responses were detected by ICS assay in all of the 15 infants and 11 mothers tested at 6–10 weeks, varying between 0.1 and 6.62% in the infants and 0.1 and 4.89% in the mothers (Fig. 1a and c Fig. 1). CD8⁺ T cell responses were higher in frequency and in magnitude than CD4⁺ T cell responses for both the infants and their mothers. Nef (67%) and Reg (60%) were the most frequently targeted CD8⁺ T cell responses in the infants, followed by Gag-specific (47%), Env-specific (33%) and Pol-specific CD8⁺ T cell responses (20%). Pol-specific responses were targeted significantly less frequently (three out of 15 infants) than Nef-specific responses (10 out of 15 infants) ($P = 0.025$) (Fisher exact test). Pol (91%) and Gag (82%) peptide pools elicited responses most frequently in the mothers, followed by Nef (64%), Reg (45%) and Env (45%) peptide pools. There was no significant difference in the magnitude of response between any of the peptide pools analyzed. These results indicate that different protein regions are more targeted by CD8⁺ T cells of 6–12-week-old perinatally infected infants than by CD8⁺ T cells of their mothers. However, the only peptide pool in which there were significantly more responses in the mothers than in the infants was Pol, as the infants had very few Pol-specific responses ($P = 0.001$); there was a trend to this effect in Gag ($P = 0.05$). Almost half (46%) of the infants could respond to three different regions, which is indicative of broadly directed CD8⁺ T cell responses. Although a larger number of mothers (82%) had CD8⁺ T cell responses to three or more peptide pools, this was not significant. A positive correlation between the infants' Env-specific ($r = 0.621$; $P = 0.014$) CD8⁺ and CD4⁺ T cell responses was observed (data not shown).

HIV-specific CD4⁺ T cell responses were detected in five of the 15 infants and eight of the 11 mothers, ranging between 0.11 and 0.54% in the infants and 0.16 and 0.84% in the mothers (Fig. 1b and d). Similarly to the CD8⁺ T cell responses, CD4⁺ T cell responses to

Nef peptide pools were most frequently detected in the infants (27%), whereas responses to Gag, Reg and Env peptide pools were all equally elicited (13%), with no responses to Pol. Gag-specific responses were the most frequently targeted in the mothers (36%), followed by Env (27%), Pol, Nef and Reg (18%). In contrast to the CD8⁺ T cell responses, which were broadly directed, the CD4⁺ T cell responses for both the infants and their mothers were much more narrowly restricted. Three of the five infants and six of the eight mothers who had a CD4⁺ T cell response responded to only one region.

No significant correlation was observed between the infants' or mothers' viral load and CD4⁺ or CD8⁺ T cell responses. There was a negative correlation between the mothers' CD4⁺ T cell count and CD8⁺ T cell responses to Env ($r = -0.669$; $P = 0.034$).

Variable peptide pools contribute most to infant's CD8⁺ T cell response

Nef-specific CD8⁺ T cell responses were dominant (of highest magnitude relative to other region-specific responses) in just over half of the infants (53%), followed by Gag-specific CD8⁺ T cell responses (20%), Reg-specific and Env-specific CD8⁺ T cell responses (13%). Pol-specific CD8⁺ T cell responses were never dominant. In the mothers, the dominant responses were distributed among the peptide pools; Gag and Pol (27%), Nef and Env (18%) and Reg (9%).

To investigate whether the infants' CD8⁺ T cell responses preferentially target variable peptides, like has been seen among acute infection in adults [20], the HIV-1-specific CD8⁺ T cell responses were divided into two response groups according to their amino acid variability. The responses to each peptide pool were calculated as a percentage of the total response (to all peptide pools) and separated into conserved (Gag and Pol) and variable proteins (Nef, Reg and Env). Responses to the more variable proteins dominated in the newly infected infants comprising an average 74% of the total response. In contrast, CD8⁺ T cell responses in the chronically infected mothers were almost equally divided between the variable and conserved proteins (Fig. 1e).

Similarity between infants and mothers in CD8⁺ and CD4⁺ T cell targeting

There was similarity between the peptides targeted by CD8⁺ T cells between mothers and their infants (Table 2 Table 2). One mother–infant pair (M/C 575) targeted three of the same peptide pools, whereas six mother–infant pairs targeted two of the same peptide pools (M/C 37, 246, 282, 331, 542 and 578). Four mother–infant pairs targeted one peptide pool of the same (M/C 235, 279, 639 and 671). By contrast, on evaluation of the peptide pools targeted by the CD4⁺ T cells, only one of the 11 mother–infant pairs shared a peptide pool target (M/C 331).

HIV-specific T cell responses at birth

Four infant samples were collected at birth, and of these one had a follow-up sample collected at 6 weeks. Two infants were infected *in utero* and two intrapartum. As expected, neither of the two intrapartum-infected infants had CD8⁺ or CD4⁺ HIV-specific responses at birth. Figure 2 Figure 2 a and b shows the HIV-specific T cell responses for the two in-utero–infected infants. Both of the in-utero–infected infants had CD8⁺ T cell responses (Fig. 2a) and the one had an Env-specific CD4⁺ T cell response at birth (Fig. 2b). Figure 2 c and d show the CD8⁺ and CD4⁺ T cell responses for the infant with birth and 6-week results. This infant had detectable Nef-specific and Reg-specific CD8⁺ T cell responses, but had no detectable CD4⁺ T cell responses at birth. At 6 weeks, Nef-specific and Reg-specific CD8⁺ T cell responses were still present but had increased in magnitude from 0.45 to 1.7% for Nef-specific and from 0.45 to 1.58% for Reg-specific responses. In addition, Pol-specific

and Env-specific CD8⁺ T cell responses and Nef-specific, Reg-specific and Env-specific CD4⁺ T cell responses could now be detected.

Can timing of transmission be determined from CD8⁺ T cell responses?

In order to predict whether the infants whose timing of transmission was unknown were infected in-utero or intrapartum, we ranked known in-utero–infected and intrapartum–infected infants separately in the order from those with the largest response to the conserved peptide pools (Gag/Pol) to those with the largest response to the variable peptide pools (Env/Reg/Nef). As variable peptides are preferentially targeted by CD8⁺ T cells in early infection [20] and then decline in chronic infection while the CD8⁺ T cell responses to the more conserved epitopes increase, we hypothesized that the longer the infants had been infected with HIV-1, the greater the contribution of conserved (and the smaller the contribution of variable) peptide pools to the total CD8⁺ T cell response. Based on this hypothesis, Fig. 3a Fig. 3 is divided into two panels: the left panel shows the known in-utero–infected infants and the right panel shows the known intrapartum–infected ones. The in-utero–infected infant placed first in the figure (C235) has 100% of its CD8⁺ T cell response directed toward the conserved peptide pools and the intrapartum infant (C279) placed last in the figure has 100% of its responses directed against the variable peptide pools (consistent with our hypothesis). Three of five in-utero–infected infants have predominantly (>50%) conserved responses and five of five intrapartum–infected infants have predominantly variable responses. Two in-utero–infected infants (C331 and C282) have predominantly variable responses (inconsistent with our hypothesis), possibly reflecting differences in disease progression. Ranking the children of unknown status similarly, we hypothesize that most are intrapartum with at most three possibly being in utero (Fig. 3b).

Discussion

The study of HIV-specific CD8⁺ and CD4⁺ T cell responses in infants has been difficult due to the large volumes of blood required for such research. As a result, only one study to date has addressed the breadth, timing and magnitude of these responses [15,16]. In this study, we have used a whole blood ICS assay to simultaneously analyze CD8⁺ and CD4⁺ T cell responses. This method overcomes the need for large sample volumes, as only 200 µl whole blood is required for each peptide pool to be evaluated.

Here, we evaluated and compared HIV-1-specific CD4⁺ and CD8⁺ T cell responses in 18 perinatally infected infants and 14 of their mothers. Both the magnitude and the breadth of the CD8⁺ T cell response were higher than that of the CD4⁺ response for the mothers and their infants. These results are in agreement with that of other studies [21–26]. Not unexpectedly, the magnitude and breadth of the mothers' T cell responses were higher than the infants' T cell responses. However, almost half of the infants had HIV-specific CD8⁺ T cell responses to three different peptide pools. Although this is fewer than the number of peptide pools that the mothers' could respond to, it is nonetheless indicative of broadly directed CD8⁺ T cell responses.

Nef-specific T cell responses were present in the majority of infants in this study. To our knowledge, this report is the first to describe Nef-specific T cell responses in perinatally infected infants and differs from another study [15] that found that Env-specific responses predominated in early infected infants. The differences observed may be due to differing methodologies, as we used a whole blood ICS assay, whereas Thobakgale *et al.* used isolated PBMCs in an IFN-γ ELISPOT assay. The latter study also had a heterogeneous intrapartum group, as the majority of their infants would have been breastfed, whereas the infants in this study were not breastfed, allowing us to define them as intrapartum or *in utero* infected, or either in the case of those with unknown timing of transmission. Both the route

of transmission and the extent of exposure to HIV-1 (expected to be persistent in the case of breastfeeding) are very likely to affect the temporal development and the magnitudes of HIV-specific T cell responses in early life. Nef-specific T cell responses have been shown to correlate negatively with age in HIV-1-infected children, and older children were found to have weak Nef-specific responses [27]. This may be due to more rapid and consistent escape mutations in Nef than in Gag or reverse transcriptase [28]. The preferential targeting of Nef by the infants in this study may be a result of large amounts of Nef protein synthesized during HIV-1 infection. Nef enhances infectivity by downregulating CD4 [29] and MHC class I receptors [30,31], suppresses both Fas and tumor necrosis factor α receptor-mediated apoptosis [32], and alters a number of intracellular signal transduction pathways.

Significant differences were observed in the patterns of CD8⁺ T cell responses to conserved proteins relative to variable proteins in perinatally infected infants and their mothers. CD8⁺ T cells responses to variable proteins comprised of a significantly larger proportion of the total response in perinatally infected infants compared with their mothers ($P = 0.045$). These results suggest similarities in T cell repertoires between perinatal infection in infants and early infection in adults [20,33-37].

We detected CD8⁺ T cell responses at birth in in-utero-infected infants, confirming the results of a recent study [15]. Additionally, CD4⁺ and CD8⁺ T cell responses were performed on one infant at birth and 6 weeks. An increase in the magnitude of the CD8⁺ T cell responses from birth to 6 weeks was observed, as well as the additional targeting of Pol and Env peptides. No CD4⁺ T cell responses were observed at birth in this infant, but Nef-specific, Reg-specific and Env-specific CD4⁺ T cell responses were observed at 6 weeks. Although anecdotal, the results are in agreement with those from other studies [38] showing that the magnitude of HIV-1-specific T cell responses increases during the first 12 months. This infant was not breastfed; therefore, increases in the T cell responses could be due to additional antigenic exposure during delivery or to maturation of the infants' immune system.

We did not observe significant correlations between the magnitude of CD8⁺ or CD4⁺ T cell responses and viral load in infants or their mothers. This may be due to small sample size or the high variability of viral load in newly infected infants. In our previous studies of larger numbers of transmitting and nontransmitting mothers combined, significant negative correlations between CD4⁺ and CD8⁺ T cell responses and viral load were observed [26].

Our data demonstrate that HIV-specific CD8⁺ and CD4⁺ T cell responses can be detected in perinatally infected infants as early as 6–10 weeks of age (CD8⁺ responses at birth in in-utero-infected infants), and that CD8⁺ T cell responses predominantly target the variable proteins as occurs in acute infection in adults. Studies of HIV-specific CD8⁺ T cell responses and viral evolution in early infection in infants suggest that such T cell responses are indeed functional and can exert selective pressures *in vivo* [39,40]. These data contribute to understanding immune response capability in the context of HIV infection in early life, and it is this capability of the adaptive immune response that is promising for the design and development of HIV vaccines for infants.

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Fig. 1.

The total magnitude of the CD8⁺ (a and c) and CD4⁺ T cell responses (b and d) is shown for 15, 6-week-old infants and 11 mothers [infants (a) and (b); mothers (c) and (d)]. gr1

The stacked bars show the contribution to the response made by each peptide pool. (e) The HIV-1-specific CD8⁺ T cell responses within each peptide pool were calculated as a percentage of the total response (to all peptide pools) and separated into conserved proteins, Gag and Pol, and variable proteins (Nef, Reg and Env). Perinatally infected infants (15) are represented by the filled boxes and the mothers (11) are represented by the open boxes. Data are presented as medians with interquartile ranges. Significant differences are indicated.

Fig. 2. HIV-1-specific T cell responses at birth and 6 weeks. gr2
The magnitudes of CD8⁺ (a) and CD4⁺ T cell responses (b) for each of the peptide pools are shown for two in-utero-infected infants at birth and the magnitudes of HIV-1-specific CD8⁺ (c) and CD4⁺ (d) T cell responses for each of the peptide pools for one in-utero-infected infant at birth and at 6 weeks.

Fig. 3.

The HIV-1-specific CD8⁺ T cell response from each peptide pool was calculated as a percentage of the total response (to all peptide pools) for the perinatally infected infants. gr3 The nine infants (tested 6–10 weeks of age) with a known timing of transmission are shown in panel (a), and the six infants whose timing of transmission is unknown are shown in (b). The infants are arranged in order from those with 100% of their CD8⁺ T cell response targeting conserved peptide pools (Gag/Pol) to those with 100% of their CD8⁺ T cell response targeting variable peptide pools (Env/Reg/Nef). The infants whose timing of transmission was unknown were predicted to be either in-utero/intrapartum or intrapartum (depending on their targeting of conserved and variable pools) as shown in the lower boxes.

Table 1

Characteristics of 14 transmitting mothers and their infected infant pairs, plus four infected infants whose mothers could not be tested.

	[0,1-2]Mother-infant pair	Timing of transmission	Time points	Mothers' CD4 cell count	Viral load (RNA copies/ml)	Maternal NVP
6	Infant	IU	Birth		229 500	
	Mom			195	491 000	-
37	Infant	IU	6 weeks		1 662 000	
	Mom			ND	78 900	+
235	Infant	IU	10 weeks		2 676 000	
	Mom			648	16 900	-
246	Infant	IU	10 weeks		2 124 000	
	Mom			341	54 400	+
282	Infant	IU	10 weeks		1 170 000	
	Mom			170	466 000	+
331	Infant	IU	Birth			
	Mom					+
331	Infant	IU	6 weeks		1 009 200	
	Mom			647	16 700	+
19	Infant	IP	Birth		-	
	Mom			253	63 800	-
43	Infant	IP	Birth			
	Mom			81	460 000	+
128	Infant	IP	10 weeks		169 200	-
	ND			511	836	
175	Infant	IP	10 weeks		864 000	
	ND			312	5820	+
187	Infant	IP	10 weeks		37 800	
	ND			378	8680	+
279	Infant	IP	10 weeks		528 600	
	Mom			219	55 600	-
540	Infant	Unknown	10 weeks		178 200	

[0,1-2]Mother-infant pair	Timing of transmission	Time points	Mothers' CD4 cell count	Viral load (RNA copies/ml)	Maternal NVP
ND			777	<400	NVP, 3TC, stavudine
542	Infant	Unknown	10 weeks	792 000	
	Mom		391	3870	+
575	Infant	Unknown	10 weeks	70 200	
	Mom		299	<400	+
578	Infant	Unknown	10 weeks	978 000	
	Mom		483	73 200	+
639	Infant	Unknown	10 weeks	20 160	
	Mom		609	408	+
671	Infant	Unknown	10 weeks	130 800	
	Mom		1011	403	+

3TC, lamivudine; d4T, stavudine; IP, intrapartum; IU, *in utero*; NVP, nevirapine.

Table 2

Comparison of HIV-specific CD8⁺ and CD4⁺ T cell responses for 11 infant–mother pairs.

		[0,3-7]CD8 ⁺ T cells						[0,8-12]CD4 ⁺ T cells					
		Gag	Pol	Nef	Reg	Env	Gag	Pol	Nef	Reg	Env		
37	Infant	+			+								
	Mom	+	+	+	+		+			+			
235	Infant	+											
	Mom	+	+	+									
246	Infant	+	+	+	+								
	Mom	+	+										
279	Infant			+					+				
	Mom	+	+	+	+	+				+			
282	Infant			+	+				+				
	Mom		+	+				+					
331	Infant		+	+	+				+	+	+		
	Mom	+	+			+					+		
542	Infant	+		+	+		+						
	Mom	+	+			+							
575	Infant	+	+	+	+				+	+			
	Mom	+	+	+	+	+					+		
578	Infant		+	+	+				+				
	Mom	+	+	+	+				+				
639	Infant			+	+								
	Mom			+	+		+						
671	Infant				+						+		
	Mom	+	+		+								

Shading indicates concordant mother–infant responses. +, a positive response.