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Continuous improvement in the immune system of HIV-infected children on prolonged antiretroviral therapy

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Abstract

Background—The goal of HAART is to promote reconstitution of $CD4^+$ T cells and other immune responses. We evaluated the extent and the kinetics of immune reconstitution in HIV-infected children over 144 weeks of successful HAART.

Methods—Thirty-seven children receiving their first HAART regimen had plasma HIV RNA; T cells and subpopulations; T-cell rearrangement excision circles (TREC) DNA; candida, HIV_{CD4} and HIV_{CD8} enzyme-linked immunospot measured at regular intervals.

Results—Plasma HIV RNA became undetectable in 81% of patients at 24 weeks and remained undetectable in 77% at 144 weeks. In contrast, $CD4^+$% continuously increased. Distribution of T-cell subpopulations changed rapidly during the first 48 weeks of HAART and more slowly thereafter. At 144 weeks, total, naive and activated $CD4^+$% and naive $CD8^+$% of HIV-infected children were not significantly different from those of healthy age-matched controls, whereas total and activated $CD8^+$% remained elevated. $CD4^+$ and $CD8^+$ TREC content increased only during the first 48 weeks of HAART. They positively correlated with each other and with total $CD4^+$%, naive $CD4^+$% and naive $CD8^+$%. Candida and HIV_{CD4} enzyme-linked immunospot increased over time reaching peak values at 48 weeks and 144 weeks, respectively. HIV_{CD8} enzyme-linked immunospot decreased in magnitude over 144 weeks of HAART but retained its breadth. Baseline $CD4^+$% positively correlated with $CD4^+$% and with functional immune reconstitution at week 144, whereas baseline TREC correlated with TREC at week 144.

Conclusion—HIV-infected children acquired normal distribution of $CD4^+$ T cells and other subpopulations and recovered CD4-mediated HIV immunity after 144 weeks of HAART.

Keywords

candida; cell-mediated immunity; children; highly active antiretroviral therapy; HIV; T-cell rearrangement excision circle; T-cell subpopulations

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Introduction

HAART significantly decreases and many times suppresses HIV replication, thus promoting recovery of $CD4^+$ T-cell numbers and other immunologic functions [1,2]. HAART, however, is also associated with numerous side effects and antiviral resistance, and, therefore, the current recommendations are to use HAART sparingly. Initiation of HAART is guided by $CD4^+$ T-cell numbers in HIV-infected adults [3]. In children, both the optimal time to initiate HAART and the best laboratory assay to determine such a time still need to be defined [4,5].

Several immunologic parameters reflecting critical functions of the immune system maybe used to assess and/ or predict immune recovery or preservation in response to HAART. The interest in the effect of HAART on the thymic output, which is commonly measured by the abundance of T-cell rearrangement excision circles (TREC) [6,7], derives from the assumption that it contributes to the expansion of the $CD4^+$ T cells [8,9]. Conversely, T-cell activation is considered a critical contributor to $CD4^+$ T-cell consumption during HIV infection [10].

Recovery of immune functions in response to HAART varies with different pathogens [11,12]. Candida-specific immunity is one of the earliest to recover, which is consistent with the disappearance of clinical signs in response to HAART [12,13]. HIV-specific CD8-cell-mediated responses tend to decline with HAARTwhereas CD4-cell-mediated responses have infrequently been demonstrated in chronic HIV infection [14-16]. Both $CD4^+$ and $CD8^+$ T-cell-mediated anti-HIV responses have been associated with control of viral replication in different stages of HIV infection [17-19]. HIV-specific $CD8^+$ T cells inhibit HIV replication *in vitro* [20-24]. HIV-specific $CD4^+$ T-cell immune responses are regularly demonstrated in long-term non-progressors and have been associated with protection against disease progression [25,26]. Furthermore, individuals who demonstrate $CD4^+$ T-cell-mediated anti-HIV responses during acute retroviral infection have a good long-term prognosis with respect to disease progression [27].

In this study, we examined the changes in TREC, T-cell subpopulations and functional cell-mediated immunity in children who started their first HAART regimen. We evaluated correlates of these immunologic parameters with control of viral replication, increase of $CD4^+$ T cells and recovery of T-cell function.

Patients and methods

Study design

The study, approved by local institutional review boards (IRB), enrolled 3-21-year-old HIV-1 infected children and adolescents into two cohorts: 3-12 and 13-21 years of age. All patients were infected perinatally. The children were either antiretroviral therapy naive or had limited exposure (≤56 days of perinatal prophylaxis or <7 days of cumulative antiretroviral treatment). Patients were required to have plasma HIV-1 RNA of at least 5000 copies/ml at entry. All children received emtricitabine, didanosine and efavirenz once daily [28]. Children discontinued study participation if they developed severe toxicity or virologic rebound defined by plasma HIV RNA of at least 1000 copies/ml on two consecutive measurements. Immunologic assays were performed at weeks 0, 24, 48, 144 or end of study if different from 144 weeks.

TREC assay

TREC were measured using a real-time PCR amplification and laser detection (Taqman) assay. $CD4^+$ and $CD8^+$ T cells from ethylene diamine tetraacetic acid-anticoagulated blood

were purified using Rosette-Sep technique (StemCell Technologies, Vancouver, British Columbia, Canada). DNA, extracted from 50 000 $CD4^+$ or $CD8^+$ cells using Qiagen (Hilden, Germany) blood columns, was amplified with a PCR primer pair specific for TREC alongside serial dilutions from 20 to 2 000 000 copies of a TREC standard [6] and negative controls using a Taqman 3700 apparatus (PE Biosystems, Foster City, California, USA). The TREC copy number in each sample was calculated by interpolation on the standard curve, and median results were reported as TREC/million peripheral blood mononuclear cell (PBMC).

Enzyme-linked immunospot (ELISPOT) assays were performed as previously described [14] using candida antigen (Greer), aldithriol-inactivated HIV-1 antigens [29] (which measured predominantly $CD4^+$ T-cell-mediated responses), and HIV-1 Gag, Pol, Nef and Env peptide pools [National Institute of Health (NIH) reagent repository], which measured predominantly $CD8^+$ T-cell responses. The peptide pools consisted of 15-mer overlapping by 11 at final concentrations of 2 μg/ml. In order to accommodate all the peptides, we used two pools each for Gag and Pol and single pools for Nef and Env. Results were expressed as spot forming centers (SFC)/1×10^6 PBMC of antigen-stimulated wells after subtraction of SFC in unstimulated wells. Positive results were defined by at least 20 SFC/1×10^6 PBMC for candida or inactivated HIV virion and at least 100 SFC/1×10^6 PBMC for HIV peptide-stimulated wells after subtraction of background, provided there was a at least two fold increase in SFC in antigen-stimulated wells compared with background.

T-cell-immunophenotyping was performed as per the pediatric and adult AIDS Clinical Trials Group consensus protocol (http://pactg.s-3.com/immlab.htm) using fluorescently labeled anti-CD4, CD8, CD45RA, CD62L, CD28, CD95, CD38 and HLADR monoclonal antibodies (Becton Dickinson, Franklin Lakes, New Jersey, USA). Results are presented primarily as percentage, because absolute numbers vary with age in the pediatric population.

Statistical analyses

Comparison of immunological responses (T-cell distribution and functional immune responses) from time on study to baseline and comparison of the two age cohorts for different responses were analyzed using the Wilcoxon signed-rank test. Normative data from HIV-uninfected children and phenotypic distribution of $CD4^+$ and $CD8^+$ T cells from the P1021 study population were analyzed by *t*-tests and stratified *t*-tests. Linear regression was used to investigate correlations of TREC data with T-cell responses.

Results

Baseline characteristics

The study enrolled 37 children, including 17 girls; five white non-Hispanic, 23 black non-Hispanic and nine Hispanic participants; with a median age of 10.5 years including 21 children less than 12 years of age. Baseline $CD4^+$ T cells and plasma HIV RNA values were 17% (range, 1-40%) and 4.7 $\log_{10}$ copies/ml (range, 2.6-6.4 $\log_{10}$ copies/ml), respectively.

Changes in HIV RNA and $CD4^+$% in response to HAART

The plasma HIV RNA rapidly decreased and became undetectable (<50 copies/ml) in 81% of the patients at week 24 (Fig. 1a). At week 144, a similar proportion of patients, 77%, had undetectable plasma HIV RNA.

The $CD4^+$% significantly increased at each visit compared with the immediately preceding one (Fig. 1b). The $CD4^+$ cell numbers increased proportionally to the $CD4^+$% from a median of 310 cells/μl at baseline to 703 cells/μl at week 144 of HAART. $CD4^+$ cell

numbers vary with the age of children, whereas $CD4^+$% does not. For this reason, the $CD4^+$% was used as the main parameter in correlation analyses. The $CD4^+$% at baseline was inversely correlated with the plasma HIV RNA (P=0.02) but not at subsequent time points. At all time points during treatment, the $CD4^+$% increased with higher baseline $CD4^+$% ($P \leq 0.02$), whereas the time on study to $CD4^+$ percentage of at least 25 decreased with higher baseline $CD4^+$% (P=0.001). Patients with higher baseline $CD4^+$% also tended to increase their $CD4^+$% by at least 5% more rapidly (P=0.07). There were no appreciable differences in virologic or immunologic responses between the two age groups at any time point.

Changes of T-cell subpopulations in response to HAART

The studies of the T-cell phenotypic distribution showed that naive $CD4^+CD45RA^+CD62L^+$% increased during the first 48 weeks of HAART (P=0.03) and remained stable thereafter (Fig. 2a). This was confirmed by analysis of the $CD4^+CD28^+CD95^-$% over time, which showed similar kinetics (not depicted). The memory $CD4^+CD45RA^-$% decreased during the first 48 weeks of HAART (P=0.02, not depicted) but not thereafter. There were no significant changes over time of HAART in the central memory $CD4^+CD28^+CD95^+$% (Fig. 2e). Activated $CD4^+CD38^+$HLADR+% decreased during the first 48 weeks of HAART (P<0.0001) but not thereafter (Fig. 2b). Because the $CD4^+$ T-cell number dramatically increased with HAART, the number of all $CD4^+$ T-cell subpopulations also increased with the exception of the $CD4^+CD38^+$HLADR+ cells, whose absolute number did not change appreciably between baseline and week 144. There were no appreciable differences between the two age cohorts with respect to changes in $CD4^+$ T-cell subpopulations over time.

In contrast to $CD4^+$ T-cell changes, which occurred mostly during the first year of HAART, $CD8^+$ T cells and subpopulations were continuously remodeled during the entire 144 weeks of observation. Naive $CD8^+CD45RA^+CD62L^+$% (Fig. 2c) and $CD8^+CD28^+CD95^-$% (not depicted) increased from baseline to week 144 of HAART (P<0.001), whereas memory $CD8^+CD45RA^-$ or $62L^-$% continuously decreased (P<0.001; not depicted). Activated $CD8^+CD38^+$HLADR+ (Fig. 2d) continuously decreased over the entire period of observation (P<0.001). The absolute cell numbers of the $CD8^+$ T-cell subpopulations mirrored the changes in the corresponding percentages. There were no appreciable differences between the two age cohorts with respect to the phenotypic changes of $CD8^+$ T cells in response to HAART.

To determine the extent to which HAART improved the distribution of T-cell subpopulations, we compared selective $CD4^+$ and $CD8^+$ T-cell subpopulations of our study participants with normative data from HIV-uninfected children [30]. The age-adjusted comparison showed that before HAART, HIV-infected children had on the average two fold lower $CD4^+$% and two fold higher $CD8^+$% compared with uninfected controls (Table 1). Naive, memory and activated $CD4^+$ and $CD8^+$ T-cell subpopulations significantly differed in untreated HIV-infected children compared with uninfected controls. At week 144 of HAART, total, naive and activated $CD4^+$% of HIV-infected children were not appreciably different from those of uninfected controls. $CD4^+$ memory percentage decreased over 144 weeks of HAART in HIV-infected children but remained significantly higher than those of uninfected controls. The total and activated $CD8^+$% remained significantly elevated in HAART recipients compared with healthy controls but the $CD8^+$ naive and memory percentage normalized. These data indicate that after 144 weeks of HAART, HIV-infected children acquired a normal distribution of most $CD4^+$ T-cell subpopulations, while retaining signs of chronic infection such as high total and activated $CD8^+$% and memory $CD4^+$%.

Changes of the $CD4^+$ and $CD8^+$ T-cell rearrangement excision circle content in response to HAART

We analyzed the kinetics of DNA TREC copies/$CD4^+$ or $CD8^+$ T cells in our two age groups (Fig. 3a and b). There was a rapid at least four fold increase in the $CD4^+$ and $CD8^+$ TREC content from baseline to week 48 ($P<0.0001$ for both), after which changes occurred in small increments that did not reach statistical significance. The $CD4^+$ and $CD8^+$ TREC increases were parallel and highly associated with each other at all time points ($r^2 \leq 0.84$; $P<0.001$). Higher age was a significant determinant of lower $CD4^+$ and $CD8^+$ TREC levels at all time points ($P<0.001$). $CD4^+$ TREC values positively correlated with total and naive $CD4^+$% at all time points ($r^2 \leq 0.51$; $P<0.001$). There was a negative correlation of $CD8^+$ TREC values with $CD8^+$% ($r^2 \leq 0.1$; $P<0.0001$) at all time points, though in the first 24 weeks of therapy, naive $CD8^+CD45RA^+CD62L^+$% marginally increased with higher $CD8^+$ TREC numbers ($P=0.06$). Both $CD4^+$ and $CD8^+$ TREC values at week 144 were higher in patients with higher baseline $CD4^+$ and $CD8^+$ TREC values, respectively. The effect of the baseline TREC level on the week 144 TREC level was independent of age.

Changes in functional immune responses during HAART

Non-specific changes in $CD4^+$ T-cell-mediated immunity were measured by ELISPOT responses to *Candida albicans* antigen. The proportion of patients with detectable candida-ELISPOT increased from 61% at baseline to 96% at week 144. Reconstitution of candida-ELISPOT responses peaked at week 48 and remained stable thereafter (Fig. 4a). There were no appreciable differences in candida-ELISPOT values between the two age cohorts at any time points. Correlation analyses were used to identify determining factors of functional immune reconstitution. Candida-ELISPOT values at week 144 were positively associated with higher baseline and week 144 $CD4^+$% ($P=0.02$ at both time points) and with higher week 144 $CD4^+CD28^+CD95^+$% ($P=0.01$).

$CD8^+$ T-cell-mediated HIV-specific immunity (HIV_{CD8}) was measured by ELISPOTresponses to Gag, Pol, Env and Nef 15-mer peptides overlapping by 11 amino acids (Fig. 4b and c). HIV_{CD8} ELISPOT values continuously decreased from week 24 to 144 ($P<0.003$ at all time points compared with the immediately preceding one). However, the breadth of the HIV_{CD8} ELISPOT, measured by the number of peptide pools that elicited any response divided by the total number of pools tested, did not significantly change on HAART (Fig. 4c). After 144 weeks of HAART, all patients had measurable HIV_{CD8} ELISPOT at least to one of the peptide pools. Correlation analyses showed no significant association of the HIV_{CD8} ELSIPOT values with plasma HIV RNA. At week 48 of HAART, the decrease of HIV_{CD8} ELISPOT values positively correlated with the decrease of $CD8^+$% ($P=0.009$).

HIV-specific $CD4^+$ T-cell-mediated responses (HIV_{CD4}) were measured by ELISPOT using an inactivated whole virion preparation [29]. The number of patients with detectable HIV_{CD4} ELISPOT responses increased from 50% at baseline to 89% after 144 weeks of HAART. HIV_{CD4} ELISPOT took longer to reconstitute and were significantly different from baseline only at 144 weeks of HAART (Fig. 4d). There were no appreciable differences in HIV_{CD4} ELISPOT values between the two age cohorts at any time points. Correlation analyses showed positive associations between HIV_{CD4} and candida-ELISPOT values at all time points ($P<0.001$). Similar to candida-specific ELISPOT, week 144 HIV_{CD4} ELISPOT values increased with higher central memory $CD4^+CD28^+CD95^+$% ($P=0.01$).

Correlations of HIV replication and T-cell activation with immune reconstitution

At baseline, high levels of plasma HIV RNA copies/ml correlated with lower $CD4^+$% T cells (P=0.02). Detectable plasma HIV RNA at week 24 was associated with lower $CD4^+$ and $CD8^+$ TREC content ($P \leq 0.002$) at week 48 of HAART. Activated $CD4^+CD38^+HLADR$+% tended to increase with higher plasma HIV RNA at baseline and at 48 weeks of HAART (P=0.07). No other appreciable correlations were observed between immune reconstitution and HIV replication.

During the first year of HAART, higher percentage of activated $CD4^+CD38^+HLADR$+ was significantly associated with immune dysfunction including lower $CD4^+$% ($P<0.001$) and lower $CD4^+$ TREC content ($P \leq 0.009$). The association of activated $CD8^+CD38^+HLADR$+% with immune dysfunction was apparent only at 144 weeks of HAART, including lower $CD4^+$% (P=0.02) and lower candida-ELISPOT and HIV_{CD4} ELISPOT values (P=0.03 for both).

Discussion

Our data demonstrate that HIV-infected children undergo progressive immune reconstitution in response to HAART that could potentially lead to normalization of immune parameters. Previous studies showed immunologic improvement in HIV-infected children and adults during the first year of HAART [31-33] and continuous increase of $CD4^+$ T cells over 6 years of therapy in children and adults who maintained undetectable plasma HIV RNA [34-36]. Here, we extend these observations by showing that not only $CD4^+$ T cells, but also functional and phenotypic immune measures continue to improve in HIV-infected children over 3 years of effective HAART. A unique feature of immune reconstitution in our study was that after 3 years of HAART, the $CD4^+$% of HIV-infected children was similar to those of healthy age-matched controls. Furthermore, the reconstitution of $CD4^+$ T cells did not differ appreciably between the two age cohorts of 3-6 and 7-21 years enrolled in this study.

It has been long recognized that HIV infection alters the distribution of T-cell phenotypes, which may be partially reversed by HAART [31,33,37,38]. However, this study is the first one to demonstrate complete normalization of T-cell subpopulations including naive and activated $CD4^+$ and naive CD8 T cells. The activated and total $CD8^+$% remained elevated, which may be due to persistent low-level viremia that can be demonstrated even in patients with plasma HIV RNA less than 50 copies/ml [39]. However, as changes in the total and subpopulations of $CD8^+$% were still actively occurring at week 144 of HAART, further improvement, leading perhaps to normal $CD8^+$% after more than 3 years of HAART, could not be ruled out.

The robust reconstitution of T cells and their subpopulations demonstrated in this study may derive from the large thymic reserve associated with the relatively young age of our study participants. We assessed thymic responses to HAART by the TREC content of $CD4^+$ and $CD8^+$ T cells. The TREC content steadily increased in both age cohorts but was consistently higher in the younger group, indicating that the thymus, whose activity increases with younger age, was the main contributor to the TREC rebound. An alternative explanation ascribing the TREC increase to HAART-associated decrease of $CD4^+$ T-cell proliferation [40] is less likely, because it does not explain the significant increase of TREC content with age (when all other responses to HAART did not differ between age groups) nor the significant correlation between $CD4^+$ and $CD8^+$ TREC content increases. The association of $CD4^+$ with $CD8^+$ TREC increases in response to HAART suggests that HIV infection inhibits thymic activity at early stages of T-cell ontogeny, before CD4/CD8 differentiation [41].

While the de-novo generation of $CD4^+$ and $CD8^+$ T cells continuously increased during the first year of HAART, this translated into an increase of $CD4^+$ but not of $CD8^+$ T cells. This finding illustrates that the number of circulating $CD4^+$ and $CD8^+$ T cells is controlled at multiple levels, such that when HAART suppresses viral replication withdrawing the antigenic stimulus for cytotoxic T-cell lymphocyte (CTL) proliferation, HIV-specific $CD8^+$ T cells decrease leading to the contraction of the entire $CD8^+$ T-cell compartment. This is further evidenced by the strong association between the decrease of HIV_{CD8} ELISPOT values and total $CD8^+$% during the first year of HAART. Ultimately, the $CD8^+$ T-cell compartment contracts during HAART, in spite of increased thymic de-novo $CD8^+$ T-cell production.

Functional immune reconstitution is an important goal of HAART, as ultimately the ability of the immune system to protect the host against opportunistic agents is a critical prognostic factor. Previous studies have shown that the reconstitution of pathogen-specific $CD4^+$ T-cell responses differs with the microbial agent, though the mechanism that underlies this difference is not well understood [12,42,43]. Here, we confirmed rapid reconstitution of *Candida* and delayed reconstitution of HIV-specific $CD4^+$ T-cell responses. However, *Candida* and HIV_{CD4} values positively correlated with each other and with total and memory $CD4^+$% and negatively correlated with activated $CD8^+$%, suggesting that functional immune reconstitution has multiple common features.

$CD4^+$ T-cell-mediated HIV-specific immunity is a hall-mark of long-term non-progression of the infection, but it is unclear whether these responses contribute to the control of viral replication or denote immune preservation [44]. Our study was not designed to address this question, and the analysis of HIV_{CD4} ELISPOT was exploratory. However, our findings, together with previous ones [14], support pursuing this question in future studies. Finding a positive association between control of viral replication and $CD4^+$ T-cell-mediated responses to HIV may change the currently accepted paradigm that HAART has a deleterious effect on HIV-specific immune defenses.

The concept that HAART decreases HIV-specific immunity stems from the observation that HAART is associated with a decrease in HIV-specific $CD8^+$ CTL [45,46]. These cells have been shown to limit HIV replication *in vitro* [22-24,47]. The role of CTL in the control of in-vivo retroviral infection was demonstrated in animal models [21,24,48]. In humans, this subject remains controversial particularly with respect to the relative importance of the magnitude against breadth of the CTL response [19,26,49,50]. Our data confirm previous reports that effective HAART is associated with a decrease in the magnitude of HIV_{CD8} ELISPOT. However, HAART did not affect the breadth of HIV_{CD8} ELISPOT.

A goal of this study was to increase our understanding of the differential contributions of HIV replication against immune activation to $CD4^+$ T-cell dysfunction. We found significant negative correlations of plasma HIV RNA with $CD4^+$% and thymic output before and during the first year of HAART. This coincided with rapid changes in the $CD4^+$ T-cell numbers and phenotypes. Activated $CD4^+$% not only negatively correlated with $CD4^+$% and thymic output in the first year of HAART, but also tended to positively correlate with HIV plasma RNA over the same period, complicating the understanding of their role in immune suppression. Activated $CD8^+$% did not correlate with plasma HIV RNA but negatively correlated with lower $CD4^+$% and function at week 144 of HAART, when most patients had undetectable viral replication. The model that emerges is that immune recovery of HIV-infected children is a biphasic process, including an early rapid phase in which the decay of viral replication is associated with recovery of thymic activity and repopulation of the T-cell compartment and with recovery of functional responses to mitogens and some antigens. A second phase of immune recovery begins or becomes evident after 6 months to 1 year of

HAART, when viremia is stable, but low. During this phase, gains in $CD4^+$ T-cell numbers and function and redistribution of $CD4^+$ T-cell subpopulations continue at a slower rate and negatively correlate with $CD8^+$ T-cell activation. Further studies are needed to determine whether the mechanism that underlies the maintenance of $CD8^+$ T-cell activation after 3 years of HAART is the viral replication, albeit at low levels, and to identify the mediators of the immune suppression after prolonged HAART.

In conclusion, this study showed a robust immune reconstitution in HIV-infected children in response to 3 years of effective HAART, that could be best predicted by the baseline immunologic characteristics of the patients. Baseline $CD4^+$% predicted the recovery of $CD4^+$ T-cell numbers and function in response to HAART, which is in accordance with previous reports [36]. We also showed that thymic output in response to HAART increased with higher thymic function at the initiation of therapy. Although HAART has potential side effects and poses significant adherence problems, its initiation in early stages of HIV infection has clear advantages with respect to immune reconstitution.

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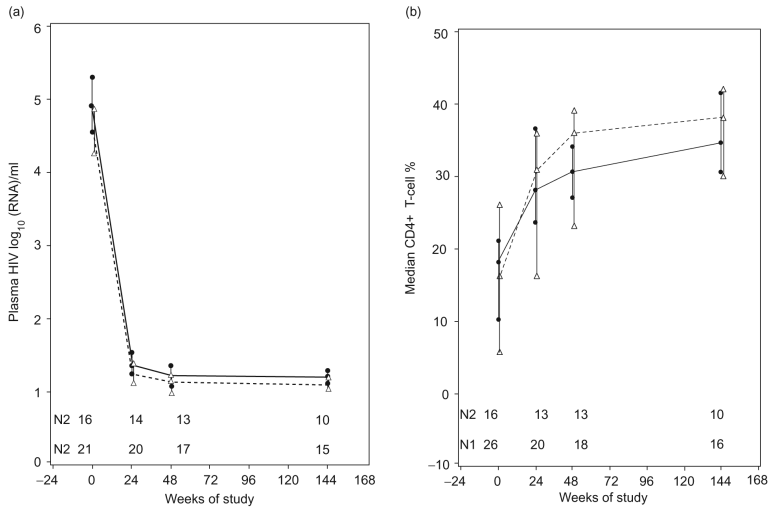

Fig. 1. Kinetics of plasma HIV RNA levels and CD4$^+$% in HIV-infected children on their first HAART regimen

(a) HIV RNA and (b) CD4$^+$%. Data represent medians, upper and lower quartiles at each time point by age group. The continuous line indicates data derived from 21 children aged 3-12 years and the interrupted line from 16 children aged 13-21 years. N_1 indicates the number of patients in the younger group that contribute data at each time point and N_2 in the older group.

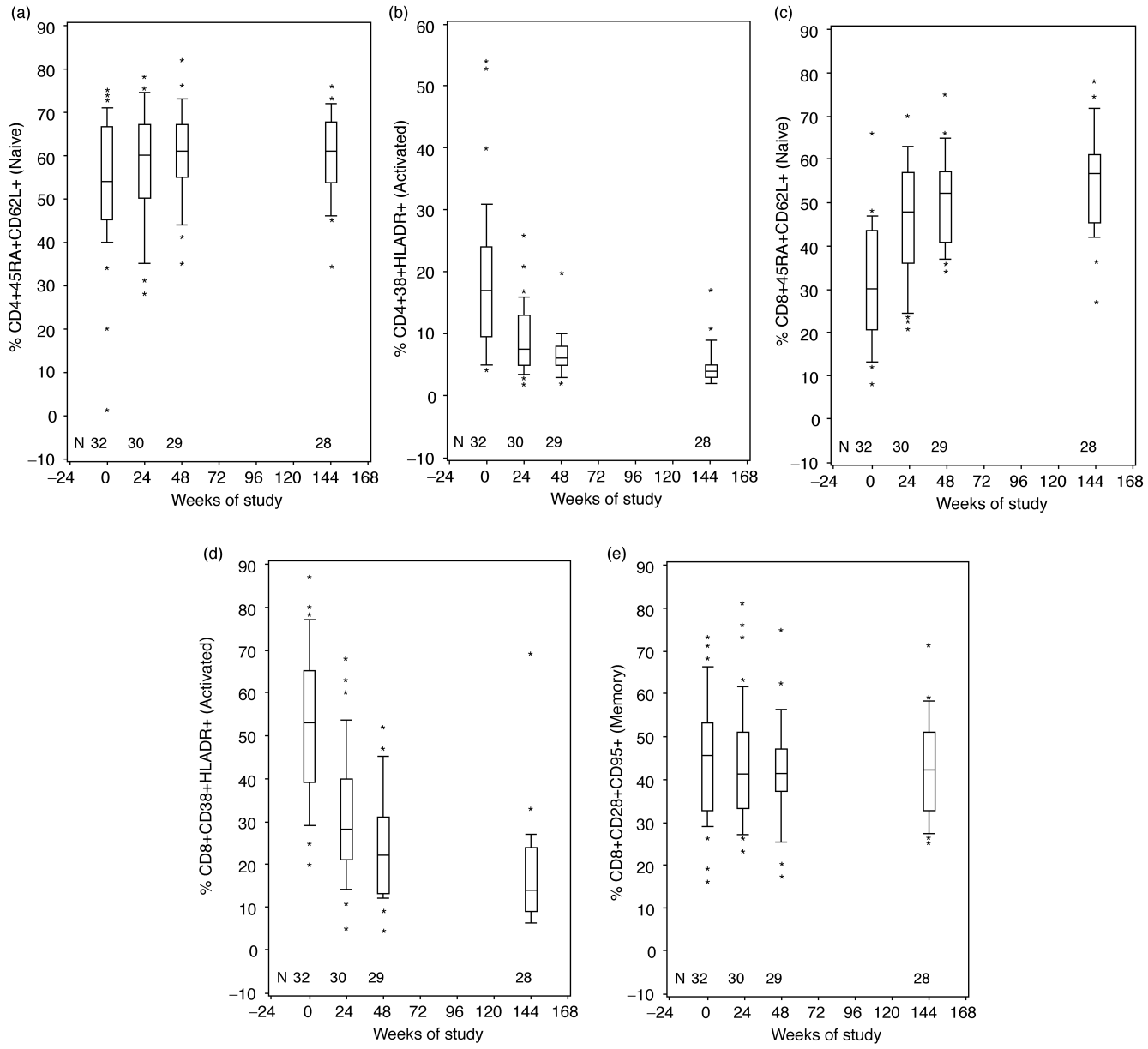

Fig. 2. Kinetics of lymphocyte subpopulations in HIV-infected children receiving their first HAART regimen

Boxes represent upper and lower quartiles with the median as an internal line; whiskers show 95% upper and lower boundaries; and asterisks indicate outliers. *N* indicates the number of patients contributing data at each time point. (a) Naive $CD4^+CD45RA^+CD62L^+$%; (b) activated $CD4^+CD38^+HLADR$+%; (c) naive $CD8^+CD45RA^+CD62L^+$%; (d) activated $CD8^+CD38^+HLADR$ %; (e) memory $CD4^+CD28^+CD95^+$% over time of HAART.

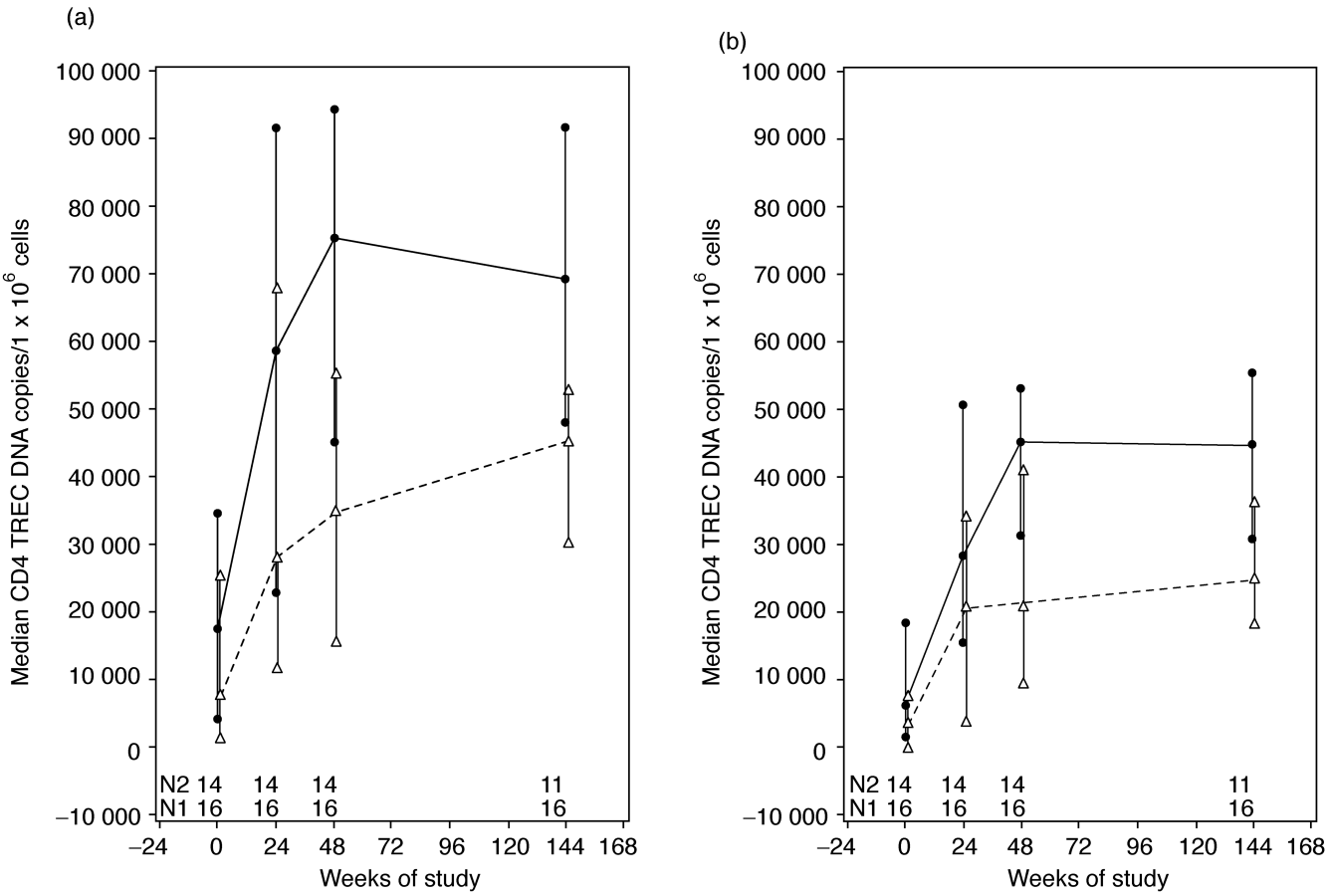

Fig. 3. Kinetics of CD4$^+$ and CD8$^+$ T-cell rearrangement excision circle content in HIV-infected children on their first HAART regimen

(a) CD4$^+$TREC; (b) CD8$^+$TREC DNA contents per 10^6 CD4$^+$ and CD8$^+$ cells, respectively. The continuous line indicates data derived from 21 children aged 3-12 years and the interrupted line from 16 children aged 13-21 years. N_1 indicates the number of patients in the younger group that contribute data at each time point and N_2 in the older group. TREC, T-cell rearrangement excision circle.

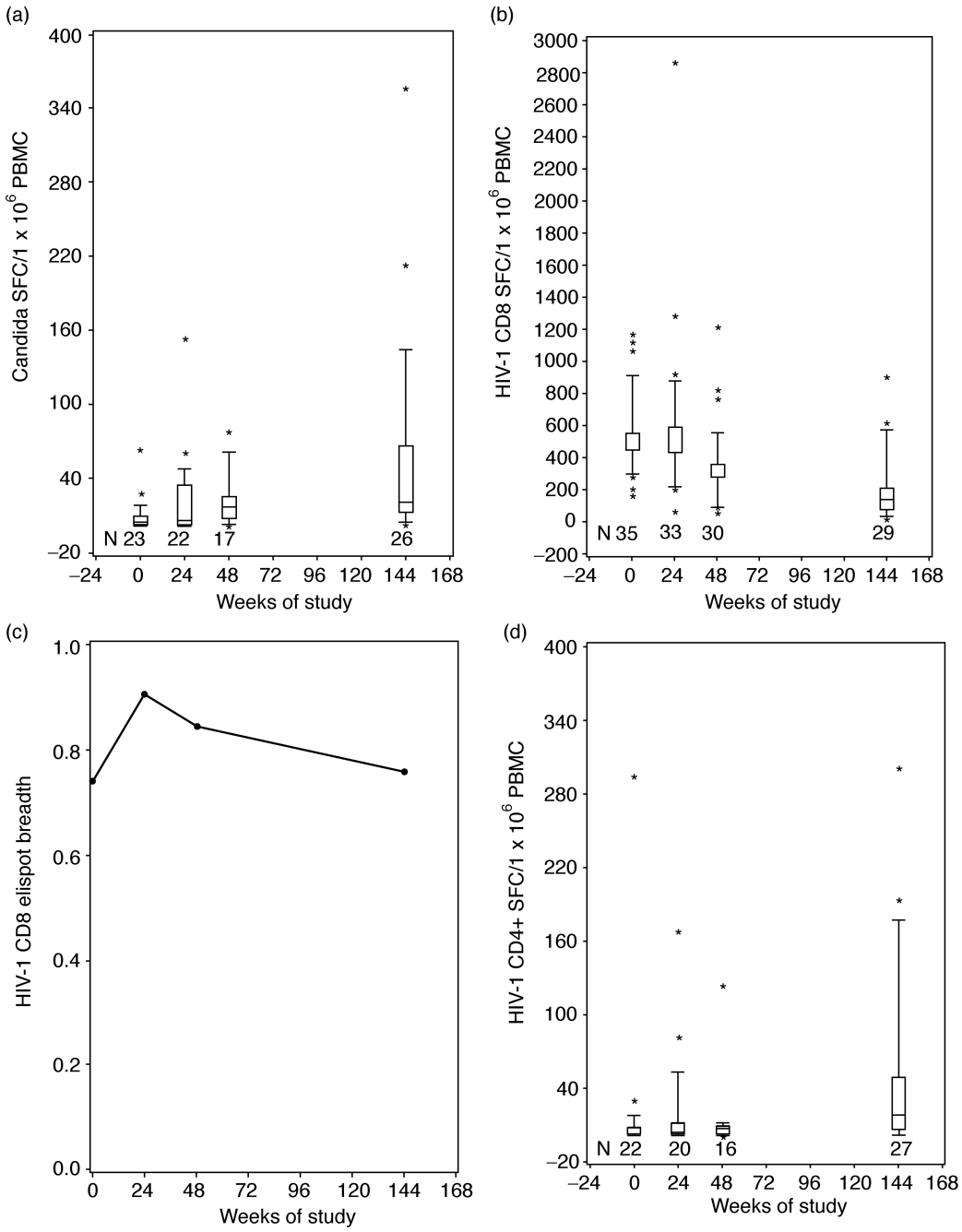

Fig. 4. Functional immune reconstitution in HIV-infected children receiving their first HAART regimen

Boxes represent upper and lower quartiles with the median as an internal line; whiskers show 95% upper and lower boundaries; and asterisks indicate outliers. *N* indicates the number of patients contributing data at each time point. (a) The time course of candida-specific ELISPOT responses; (b) CD8-mediated HIV-specific ELISPOT responses after stimulation with peptide pools derived from Gag, Pol, Nef and Env; (c) the breadth of CD8-mediated HIV-specific ELISPOT responses expressed as a fraction of the number of peptide pools that elicited any response divided by the total number of pools tested; (d) CD4-mediated HIV-specific responses after stimulation with inactivated whole HIV virion. *N*

indicates the number of patients contributing data at each time point. ELISPOT, enzyme-linked immunospot; PBMC, peripheral blood mononuclear cells; SFC, spot forming centers.

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Table 1

Distribution of T-cell phenotypes of HIV-infected children approaches normal standards after 144 weeks of HAART

Phenotype	Control[a]	Week 0[b]	*P* value[c]	Week 144[b]	*P* value[d]
$CD4^+$ total	34 ± 15[e]	17 ± 10	<0.0001	36 ± 7	0.60
$CD4^+CD28^+CD95^-$ naive	56 ± 15	46 ± 22	<0.0001	55 ± 12	0.38
$CD4^+CD28^+CD95^+$ memory	39 ± 14	45 ± 15	0.01	43 ± 12	0.05
$CD4^+CD38^+$HLADR+ activated	4 ± 4	19 ± 13	<0.0001	5 ± 3	0.66
$CD8^+$ total	22 ± 10	50 ± 13	<0.0001	31 ± 8	<0.0001
$CD8^+CD45RA^+CD62L^+$ naive	60 ± 15	31 ± 14	<0.0001	55 ± 12	0.22
$CD8^+CD45RA^-CD62L^+$ memory	42 ± 14	69 ± 14	<0.0001	45 ± 12	0.20
$CD8^+CD38^+$HLADR+ activated	12 ± 11	53 ± 18	<0.0001	17 ± 13	0.01

[a] Measures in healthy children.

[b] Measures in HIV-infected children at weeks 0 and 144 of HAART.

[c] Age-adjusted comparison of week 0 measures in HIV-infected children against uninfected controls.

[d] Age-adjusted comparison of week 144 measures in HIV-infected children against uninfected controls.

[e] Numbers represent means ± SD%.