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 Restoration of the CD4 T Cell Compartment after Long-Term Highly Active Antiretroviral Therapy without Phenotypical Signs of Accelerated Immunological Aging

Nienke Vrisekoop,*† Rogier van Gent,‡ Anne Bregje de Boer,* Sigrid A. Otto,* Jan C. C. Borleffs,§ Radjin Steingroever,¶ Jan M. Prins,§ Taco W. Kuijpers,¶ Tom F. W. Wolfs,¶ Sibyl P. M. Geelen,¶ Irma Vulto,# Peter Lansdorp,# Kiki Tesselaar,* José A. M. Borghans,*** and Frank Miedema*‡

It remains uncertain whether full T cell reconstitution can be established in HIV-infected children and adults with long-term sustained virological control by highly active antiretroviral therapy (HAART). In this study, we comprehensively analyzed various phenotypical markers of CD4 T cell recovery. In addition to measuring T cell activation and proliferation markers, CD4 T cell generation and aging of the CD4 T cell compartment were assessed by measuring TCR excision circles and the fraction of CD31-expressing naive CD4 T cells. In all children and in adults with relatively high CD4 T cell counts at start of therapy (>200 cells/µl), total CD4 T cell numbers normalized within 1 year of therapy. After long-term HAART (4.4–9.6 years), naive CD4 T cell counts had normalized in both groups. Although in adults with low baseline CD4 T cell counts (<200 cells/µl) total CD4 T cell numbers normalized eventually after at least 7 years of HAART, naive CD4 T cell counts had still not recovered. TCR excision circle data showed that thymic T cell production contributed to naive T cell recovery at all ages. The fraction of CD31-expressing naive CD4 T cells was found to be normal, suggesting that the CD4 T cell repertoire was diverse after long-term HAART. Hence, under sustained viral suppression during long-term HAART, the T cell compartment has the potential to fully recover by generating new naive T cells both in children and adults with high baseline CD4 T cell counts. Irrespective of baseline CD4 T cell counts, reconstitution occurred without a significant effect on T cell aging as reflected by markers for replicative history. The Journal of Immunology, 2008, 181: 1573–1581.

Since the introduction of highly active antiretroviral therapy (HAART) as a treatment for HIV-1 infection, many studies have investigated its effect on the reconstitution of the CD4 T cell compartment. Analyses of reconstitution in HIV cohorts, with a large variation in treatment protocols and degree of viral suppression, have shown that normalization of the T cell compartment is generally not achieved. Few studies have analyzed reconstitution in patient groups with sustained viral suppression. These studies have shown that in the majority of adult patients, CD4 T cell counts gradually increase (1, 2). Some studies report CD4 T cell counts reach a plateau after 3–5 years of HAART (2, 3); however, others found continuous CD4 T cell gains throughout 4 years of therapy (1). Total CD4 T cell numbers in adult patients with low baseline (pretherapy) CD4 T cell counts were found to remain low throughout the reconstitution period and failed to normalize within 4–5 years of HAART (1, 2). However, the net yearly increase in CD4 T cell counts was shown to be independent of baseline CD4 T cell counts (1).

Because thymic output decreases from early childhood, one would expect that immune reconstitution is less effective with increasing age (4). Indeed, increases in total and naive CD4 T cell numbers were found to be higher in younger HIV-infected children compared with older HIV-infected children on HAART (5, 6) and higher in HIV-infected children compared with adults (7). However, when normalized for naive and total T cell numbers in age-matched controls, the relative gain was age-independent (6, 8).

The importance of thymic output during reconstitution has been suggested by the association between poor CD4 T cell reconstitution during HAART and age (1, 2, 9–11), low absolute numbers of naive CD4 and CD8 T cells (10, 12), less thymic tissue (10, 11, 13–15), and low absolute numbers of TCR excision circles (TRECs) (16). TRECs are by-products of TCR rearrangements

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which are not duplicated upon cell division (17, 18). Consequently, absolute numbers of TREC per milliliter of blood can provide information on thymic output, whereas the replicative history of the T cell compartment, either by activation or homeostatic proliferation, can be determined by measuring the average TREC content per cell in conjunction with T cell numbers.

In addition to thymic output, reconstitution of the T cell compartment can be achieved by peripheral T cell proliferation. Studies in HIV-infected adults have shown that although T cell proliferation declined after initiation of therapy, it was still increased compared with healthy individuals up to 2 years after treatment initiation (19). Furthermore, CD4 and CD8 T cell activation markers had not normalized after 2–6 years of therapy (19–22). Similarly, HIV-infected children who had been on HAART for 3–4 years had significantly higher levels of activated (CD38^+) CD4 and CD8 T cells than age-matched controls (12, 19, 20, 26, 27) and proliferating (Ki67^+) naive CD4 and CD8 T cells (27) are accelerated loss of thymic output in these patients. Recently, high levels of activated CD38^+ T cells (12, 19, 20, 26, 27) and proliferating (Ki67^+) naive CD4 and CD8 T cells (27) are related to poor CD4 T cell reconstitution support the notion that residual activation rather than a homeostatic response drives T cell proliferation during HAART. In addition, the observations that low TREC contents (10–12, 16, 24, 25), short telomeres (10), and levels of activated CD38^+ "HLA-DR^+" T cells (12, 19, 20, 26, 27) and proliferating (Ki67^+) naive CD4 and CD8 T cells (27) are related to poor CD4 T cell reconstitution support the notion that residual activation causes the increased levels of T cell proliferation.

Based on immune reconstitution studies after stem cell transplantation (SCT) in which inadequate long-term T cell reconstitution, especially in adults, has repeatedly been reported (28–31), normalization of the T cell compartment within 7 years of HAART is not to be expected. However, the incomplete T cell reconstitution after SCT may be explained by the inclusion of a subset of SCT patients with poor early engrafting (32) or graft-vs-host disease (33, 34) rather than by long-term immune failure caused by accelerated loss of thymic output in these patients. Recently,
another study showed successful T cell reconstitution 17 years after chemotherapy-induced lymphopenia (35). Moreover, Dion et al. (36) showed that thymopoiesis contributed to T cell recovery during HAART. Therefore, we hypothesized that adequate T cell reconstitution may be possible in HIV-1-infected individuals on continuous treatment with HAART.

The introduction of HAART has resulted in sustained virological suppression, i.e., viral loads below the detection threshold during the course of therapy, in many HIV-infected individuals. This gave us the opportunity to study whether under sustained virological suppression during the entire treatment, full T cell reconstitution in HIV-1-infected children and adults can in principle be achieved during long-term (4.4–9.6 years) HAART, and whether this T cell reconstitution results in accelerated aging of the T cell compartment in the long run. Analysis of quantitative naive and memory CD4 T cell recovery was combined with measurements of activation and proliferation markers to establish to what extent long-term HAART had normalized these parameters. Furthermore, we assessed the replicative and postthymic history of T cells by analyzing their TREC contents and the fraction of CD31-expressing naive CD4 T cells. If the increase in CD4 T cell counts observed during HAART would mainly be due to T cell proliferation, this would lead to increased aging of the peripheral T cell pool, i.e., in dilution of the T cell TREC content and low fractions of CD31+ naive CD4 T cells. Conversely, decreasing levels of T cell proliferation in concert with the entrance of thymic emigrants into a virtually empty peripheral T cell compartment could in theory lead to rejuvenation of the peripheral T cell compartment. We show that in children and even in adults with more than 200 CD4 T cells/μl blood at start of therapy, the CD4 T cell compartment has the potential to fully recover during long-term successful HAART. Thymic naive T cell generation and possibly also peripheral naive T cell proliferation contributed to this recovery in such a way that reconstitution did not have a significant effect on T cell aging as evaluated by CD31 expression and TREC analyses.

Materials and Methods

Study population

To avoid unwanted patient selection bias, we included all HIV-1-infected children and adults (21 and 26, respectively) from the patient populations of the University Medical Center (UMC; Utrecht, The Netherlands) and the Academic Medical Center (AMC; Amsterdam, The Netherlands) who matched our selection criteria (see Table I for patient characteristics). To be eligible, they had to be treated with long-term continuous HAART (range children: 4.4–9.6 years; range adults: 7.0–9.2 years) and had to have adequate viral suppression for the complete period of follow-up, with a minimum detection threshold of 50 copies HIV-1 RNA/ml. Individuals with an occasional appearance of plasma HIV-1 RNA above the detection threshold were included in the analyses, but only if the load returned to undetectable levels within 3 mo upon detection and without modifications to HAART regimens. Thirteen adults and nine children had, on one or more occasions, plasma HIV-1 RNA copies above the detection threshold. Patient A04 had three blips, all in the first 2 years of treatment. HAART was defined either as a combination of at least three drugs from at least two different drug classes (which are: protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and the nucleotide reverse transcriptase inhibitor tenofovir) or as a combination of three nucleoside reverse transcriptase inhibitors including abacavir. No patient selection was performed on immunological parameters (Table II).

All control values of the various immunological parameters were provided by a cohort of healthy volunteers, consisting of 92 children (age range: 0.1–18.0 years) and 107 adults (age range: 19.0–65.0 years). Because T cell counts decrease during childhood, total and subset T cell counts of the healthy controls were stratified into 10 age groups to normalize T cell counts of children during HAART. These age groups were respectively: 0–0.5, 0.5–1.0, 1.0–2.0, 2.0–3.0, 3.0–4.0, 4.0–6.0, 6.0–9.0, 9.0–12.0, 12.0–15.0, and 15.0–18.0 years. Each group consisted of at least seven children.

![FIGURE 1. CD4 T cell recovery in children and adults on HAART. CD4 T cell counts as a percentage of age-matched control values (denoted by dotted horizontal lines) are illustrated from start of HAART and thereafter. Children and adults were subdivided into two groups according to a baseline CD4 T cell number of 25% of control values. Data are shown as mean ± SD. Statistical significance (∗, p < 0.05) from 100% was determined by a one-sample t test, given the assumption that the groups are derived from a population with a normal distribution, as was confirmed by the nonparametric one-sample Kolmogorov-Smirnov test.](http://www.jimmunol.org/doi/fig/1575)
This study was approved by the medical ethical committee and written informed consent was obtained from all study participants or their legal guardians in agreement with the Helsinki Declaration of 1975, revised in 1983.

Flow cytometry and cell sorting
PBMC were obtained by Ficoll-Paque density gradient centrifugation from heparinized blood and were cryopreserved until further use. Absolute counts and maturation status of CD4 T cells and immune activation status of CD4 and CD8 T cells were routinely measured. Absolute CD4 T cell counts were determined by dual-platform flow cytometry. Naive (CD27+CD45RO−) and memory (CD45RO+) CD4 T cell fractions and activated (CD38+HLA-DR+) CD4 and CD8 T cells were assessed by flow cytometry. To measure the fraction of CD31+ cells within the naive CD4 T cell population and peripheral blood T cell proliferation in CD4 T cell subsets, cryopreserved PBMC were thawed and incubated with mAb to CD45RO-FITC (Caltag Laboratories), CD31-PE, CD4-PerCP (BD Biosciences), and biotinylated CD27 (Sanquin Reagents). After washing, cells were incubated with anti-streptavidin-allophycocyanin (BD Biosciences). To measure T cell proliferation, cells were fixed (FACS lysis solution; BD Biosciences), permeabilized (FACS permeabilization buffer; BD Biosciences), and stained intracellularly with Ki67-FITC (Monosan), after which cells were resuspended in Cellfix (BD Biosciences) and were analyzed on a FACSCalibur (BD Biosciences) with CellQuest software.

To measure the TREC content within the naive (CD27+CD45RO−) CD4 T cell population, PBMC were incubated with mAb to CD45RO-FITC (Caltag Laboratories), CD27-PE, and CD4-PerCP (BD Biosciences). The specified cell fractions were isolated by cell sorting on a FACSAria (BD Biosciences). To measure the TREC content within CD4 T cells, these subsets were purified from thawed PBMC by magnetic bead separation using the MiniMACS multisort kit according to the manufacturer’s instructions (Miltenyi Biotec).

FIGURE 2. Recovery of CD4 T cell subsets in children and adults after long-term HAART. Numbers of naive (CD27+CD45RO−) and memory (CD45RO+) CD4 T cells after long-term HAART (4.4–9.6 years) expressed as percentage of age-matched control values in HIV-infected children, and adults with low or high baseline CD4 T cell counts. Adults were subdivided into two groups according to a baseline CD4 T cell number of 25% of control values. Data are shown as mean ± SD. Statistical significance was determined by the nonparametric Mann-Whitney U test: *, p < 0.05; **, p < 0.01.

FIGURE 3. Recovery of various immune parameters after long-term HAART. A, TREC content per CD4 T cell. B, Total CD4 T cell TREC numbers per microliter of blood. C, TREC content of naive CD4 T cells. D, Percentage of CD31+ cells in the naive CD4 T cell compartment. Adults were subdivided into two groups according to a baseline CD4 T cell number of 25% of control values. Lines connect data points at the start of HAART and after long-term HAART (4.4–9.6 years) within one individual. Whenever only one point is given it represents the value after long-term HAART.
TREC analysis

DNA was isolated using the QIAamp Blood kit according to the manufacturer’s instructions (Qiagen). Signal joint TREC numbers were quantified using real-time PCR as described previously (18, 37).

Mathematical model

To study the effect of HAART on the dynamics of T cell TREC contents, we used a previously developed mathematical model for TREC decline with age (18), described by the following two differential equations: \( dN/dt = \alpha + pN - dN \) and \( dT/dt = \alpha - dT \), where \( N \) is the number of CD4 T cells, \( T \) is the total number of TREC, \( \alpha = a e^{-\gamma} \) represents thymic output which declines at rate \( \gamma \) per year, and T cells proliferate at rate \( p \) and die at rate \( d \) per day. The TREC content of the CD4 T cell population was calculated as \( T/N \). It has previously been shown that the average T cell TREC content does not decrease with age if there is no homeostatic regulation of T cell numbers (38). We therefore modeled the rate of T cell proliferation as: \( p = p_{0T} + p_{0T}(1 + N^2/h^2) \), where \( p_{0T} \) represents the maximum proliferation rate in healthy individuals (which is attained when T cell numbers are very low), \( h \) is the T cell count at which the division rate is half-maximal in healthy individuals, and \( p_{0T} \) represents the extra T cell proliferation induced by HIV infection.

Results

Recovery of CD4 T cell counts in children and adults during long-term HAART

We aimed to determine whether, during sustained viral suppression, CD4 T cell counts normalized in children and adults on long-term (4.4–9.6 years) HAART, and whether children, as they may have better reconstituting capabilities, demonstrated enhanced CD4 T cell recovery compared with adults. The mean CD4 T cell count at start of HAART (baseline) was 688 cells/\( \mu \)l blood (range: 50–2274) for children and 206 cells/\( \mu \)l blood (range: 19–560) for adults. Because T cell counts per microliter of blood decrease with age and stabilize at the start of adolescence, total CD4 T cell counts, as well as counts of CD4 T cell subsets, were normalized for age-matched control values, which were derived from our cohort of healthy volunteers and were similar to values published by others (39, 40). The mean normalized CD4 T cell count number at the start of HAART was higher for children than for adults (39.5 and 25.7% of the age-matched control value, respectively). To make a comparison between T cell reconstitution in adults and children, and to evaluate the influence of baseline cell numbers on T cell reconstitution, we separately analyzed pediatric and adult patients with low and high CD4 T cell numbers at start of treatment. The value to discriminate these groups was chosen to be 25% of age-matched control values, which is equal to 200 CD4 T cells/\( \mu \)l blood in adults and delineates stages 1 and 2 vs 3 of the CDC classification system. In children with CD4 T cell counts <25% of their age-matched value at baseline, CD4 T cell counts reached similar levels within 1 year of HAART as in children who had higher baseline CD4 T cell counts (\( p = 0.80 \), Fig. 1) and in both groups CD4 T cell counts were not significantly different from age-matched values from 1 year of HAART onward. In contrast, in adults, the difference in CD4 T cell counts between the two groups remained throughout follow-up. After 1 year of HAART, CD4 T cell counts did not significantly differ from those of controls (\( p = 0.136 \)) in adults with high baseline CD4 T cell counts. CD4 T cell counts in adults with low baseline CD4 T cell counts persistently lagged behind, but eventually after at least 7 years of HAART, CD4 T cell counts were attained which did not differ from control values (\( p = 0.176 \)).

CD4 T cell subsets are perturbed in adults after long-term HAART

Although total CD4 T cell counts of children and adults had returned to normal levels after long-term HAART with sustained viral suppression, we questioned whether the naive T cell compartment had restored to normal levels and whether expansions within the memory T cell compartment had contracted. Because differences in naive and memory CD4 T cell subsets between children with high and low baseline CD4 T cell counts were not observed after long-term HAART (data not shown), the data are no longer presented separately for the two groups of children. In children, numbers of naive (CD27+CD45RO−) CD4 T cells had returned to normal levels after long-term HAART, whereas numbers of memory (CD45RO+) CD4 T cells were significantly lower than in healthy age-matched controls (\( p = 0.004 \), Fig. 2). Adults with low baseline CD4 T cell counts had normal numbers of memory CD4 T cells and lower naive CD4 T cell counts after long-term HAART compared with healthy controls (\( p = 0.001 \), Fig. 2). In contrast, in adults with high baseline CD4 T cell counts, numbers of memory CD4 T cells were higher than in healthy controls (\( p = 0.022 \)), and the naive CD4 T cell compartment had regained normal numbers (Fig. 2).

Normal CD4 T cell TREC contents and numbers after long-term HAART

Next, we studied the origin of the newly produced naive T cells during HAART and whether CD4 T cell recovery was associated with altered aging of the T cell compartment. On the one hand, increased peripheral T cell proliferation could lead to accelerated aging and reduced diversity of the T cell compartment in terms of diluted T cell TREC contents and low fractions of CD31-expressing naive CD4 T cells. On the other hand, if new T cells were only derived from thymic output, their appearance in a virtually empty T cell pool may lead to higher TREC contents and higher levels of CD31+ naive CD4 T cells compared with healthy age-matched controls (33). After long-term HAART, all children and most
adults had similar TREC contents in CD4 T cells as age-matched controls (Fig. 3A). In general, total CD4 T cell TREC numbers (Fig. 3B) as well as TREC contents of sorted naive CD4 T cells (Fig. 3C) after long-term HAART did not differ from control values either. From a number of adults, baseline CD4 T cell TREC contents and numbers were available. In adults with low CD4 T cell TREC contents at the start of HAART, an increase was observed during long-term HAART reaching (the lower end of) normal TREC contents, with the exception of two individuals who still had low TREC contents (Fig. 3A). However, all these adults showed an increase in total CD4 T cell TREC numbers during HAART (Fig. 3, A and B). This suggests that thymic T cell production contributed to the recovery of the naive CD4 T cell compartment. Thus, in terms of TREC contents, the CD4 T cell compartment did not seem to have undergone accelerated aging during long-term HAART.

**Normal fraction of CD31-expressing naive CD4 T cells after long-term HAART**

CD31⁺ naive CD4 T cells are most proximal to the thymus (41) and have been described to have a much more diverse TCR repertoire than CD31⁻ naive CD4 T cells (41, 42). During aging, the percentage of CD31-expressing naive CD4 T cells has been shown to decrease (41), raising the question of whether the fraction of CD31-expressing naive CD4 T cells, and thereby normal levels of naive T cell repertoire diversity, can be attained during immune reconstitution. To determine whether CD4 T cell recovery was associated with accelerated aging of the naive T cell compartment, the fraction of CD31⁺ cells was determined within the naive CD4 T cell compartment after long-term HAART. The fraction of CD31⁺ naive CD4 T cells after long-term HAART in both adults and children was comparable to age-matched values (Fig. 3D), which corroborates the finding that the extensive increases in CD4 T cell counts during HAART had not caused accelerated aging of the naive CD4 T cell subset.

**Mathematical model explaining the observed T cell recovery during long-term HAART**

At first glance, it may seem counterintuitive that the T cell compartment shows no signs of rejuvenation or aging, in terms of abnormal TREC values and CD31 percentages, after reconstitution of a substantially depleted CD4 T cell compartment. However, the use of a simple previously developed mathematical model for TREC dynamics (Materials and Methods) (18) shows that, in fact, this is exactly what is to be expected. In healthy individuals, T cell TREC contents reflect an equilibrium between the levels of proliferation and thymic output characteristic for the age of the individual. At higher age, when there is less thymic output, the higher
level of T cell proliferation that is required to keep T cell numbers relatively constant causes the TREC content of the T cell population to be reduced. This TREC dilution effect is accelerated during HIV infection, which is known to considerably increase T cell proliferation levels (Fig. 4, age 20) (18). When long-term HAART causes the level of immune activation to return to nearly normal levels, the CD4 T cell TREC content reverts to its normal age-matched control level, representing the equilibrium between the level of T cell proliferation and thymic output characteristic for the age of the individual (Fig. 4, age 30).

**Increased T cell division levels in adults after long-term HAART**

Because the T cell compartment had recovered to normal levels after long-term HAART, we expected T cell division to have returned to normal levels. To assess this, the expression of the proliferation marker Ki67 was measured within CD4 T cell subsets after long-term HAART. In children, naive (CD27+/CD45RO−) and memory (CD45RO+) CD4 T cells after long-term HAART indeed expressed similar levels of Ki67 as age-matched controls (Fig. 5, A and B). In both adult groups, in contrast, Ki67 expression within naive (p < 0.001) and memory (p < 0.01) CD4 T cells after long-term HAART was still increased compared with age-matched controls. No significant differences between the two adult groups were observed in terms of Ki67 expression in any of the CD4 T cell subsets after long-term HAART (Fig. 5, A and B). Nevertheless, Ki67 expression in both adult groups had declined in all T cell subsets during HAART, although only significantly within naive CD4 T cells of adults with high baseline CD4 T cell counts (p = 0.05; Fig. 5A).

**Increased levels of Ki67 expression are not related to T cell activation**

Although viral levels were below the threshold of detection, it cannot be ruled out that T cell activation by residual viral replication could cause the increased levels of proliferation observed within the CD4 T cell subsets in adults after long-term HAART. Therefore, the expression of the T cell activation markers CD38 and HLA-DR was determined on CD4 and CD8 T cells from adults. The expression of these activation markers had already normalized 1 year after initiation of HAART, except on CD4 T cells from adults with low baseline CD4 T cell counts, and remained relatively stable at normal levels during follow-up (Fig. 5, C and D). Only adults with high baseline CD4 T cell counts showed a slightly elevated expression level of activation markers on CD8 T cells after long-term HAART (p = 0.013; Fig. 5D), although after 1 year of HAART normalization was observed. No relation between the levels of activation and Ki67 expression of CD4 T cells (p = 0.216 low baseline CD4 T cells; p = 0.686 high baseline CD4 T cells; p = 0.154 grouped, data not shown) or CD8 T cells (p = 0.125 low baseline CD4 T cells; p = 0.793 high baseline CD4 T cells; p = 0.168 grouped, data not shown) was observed, suggesting that the elevated levels of T cell proliferation were not clearly associated with T cell activation. Expression levels of CD38 and HLA-DR on CD4 and CD8 T cells of children were normal after long-term HAART (data not shown).

**Discussion**

Contrary to the common belief, our analyses show that both in HIV-1-infected children and adults, which were selected for sustained viral suppression and long follow-up, reconstitution of the CD4 T cell compartment during long-term HAART can be achieved. Although it was shown recently that after 7 years of continuous HAART CD4 T cell counts in adults can reach levels of 800 cells/μl blood (43), most previously published studies failed to point out to what extent normalization of the T cell compartment occurs during successful HAART. In these studies concerning immune reconstitution during HAART, a selection was made for immunological responders and/or nonresponders (8, 10–12, 16, 25, 27, 44, 45), age-matched controls were not included (5, 7, 10, 11, 25, 27, 46–48), and mainly relatively short-term effects were studied (5–7, 10, 19, 47–50). In many of the pediatric studies, patients had been pretreated (3, 6, 12, 24, 25, 48, 49) and residual viral load often persisted (7, 11, 12, 24, 25, 48).

The fact that parameters like the fraction and absolute numbers of CD31-expressing naive CD4 T cells and T cell TREC content decrease with age suggests that, even during aging of healthy individuals, the immune system is not able to keep T cell numbers stable without generation of T cells by peripheral proliferation. During reconstitution after severe T cell depletion one may therefore intuitively expect to see signs of accelerated aging of the T cell compartment. We found, however, that after long-term HAART, the T cell TREC content and the fraction of CD31-expressing naive CD4 T cells, had all returned to the level of age-matched controls, even in adults with baseline CD4 T cell counts below 200 cells/μl. The increases in these immune parameters during HAART demonstrate that thymic output was a source of new naive T cells in children, but also in adults. This supports the data from a previous report by Dion et al. (36). In healthy individuals, the TREC content of naive CD4 T cells has been found to decline during aging (17). It was shown that density-dependent peripheral naive T cell proliferation is most likely causing this decline (38, 51). If during HAART, the thymus would be the only source of naive T cell production, one would expect that after long-term HAART TREC contents would be higher than those of age-matched healthy individuals (33). Because the TREC content in naive CD4 T cells was found to be similar to that of age-matched healthy individuals, our data suggest that in addition to thymic naive T cell production, peripheral naive T cell proliferation also contributed to the recovery of the naive T cell compartment during HAART.

The observation that the fraction of CD31-expressing naive CD4 T cells, which are cells most proximal to the thymus (42) and are characterized by a diverse TCR repertoire (41) had normalized, suggests that after long-term HAART, the naive T cell repertoire may be as diverse as in healthy age-matched individuals. Furthermore, we found that telomere length of memory CD45RA+ T cells had normalized during long-term therapy (data not shown). The restricted use of Abs in the flow-fluorescence in situ hybridization assay does not allow for staining with CD27 mAbs. Consequently, telomere length of naive T cells could not be measured as CD45RA+ T cells may be contaminated by the presence of terminally differentiated effector (CD45RA−CD27+) CD8 T cells. However, because the average telomere length of memory cells is highly linked to the telomere length of naive T cells (52), it is most unlikely that the telomere length of naive T cells was significantly shortened after long-term HAART, because memory T cells were found to have normal telomere lengths.

Although all of our data suggest successful recovery of the CD4 T cell compartment after long-term HAART, our analyses focused on quantitative reconstitution only. Functional assays will have to be performed in future research to ascertain whether the quantitative recovery of the CD4 T cell compartment that we observed by phenotypical measures also leads to qualitative reconstitution of T cell function after long-term HAART.

In agreement with previous observations by others, who studied T cell reconstitution after 18 mo of treatment (48), we found that children with low baseline CD4 T cell counts recovered to similar levels of CD4 T cells as children with higher baseline counts.
within 1 year of HAART, and that from 1 year of therapy onward both groups had no significantly lower CD4 T cell counts compared with healthy age-matched values. Furthermore, numbers of naive CD4 T cells had normalized in children on long-term HAART. Hence, these data suggest that postponing the initiation of treatment of HIV-infected children, which may be beneficial to avoid unwanted side effects of HAART, may not interfere with T cell reconstitution during HAART.

Although patient numbers are limited in this study, CD4 T cell counts after long-term HAART normalized in both adults with low and high baseline CD4 T cell counts. Apparently, the occurrence of one or two viral blips observed during the entire follow-up in a number of patients did not seem to interfere with the recovery of CD4 T cell counts. However, the number of naive CD4 T cells after long-term HAART did not normalize in adults with low baseline CD4 T cell counts and full phenotypical CD4 T cell reconstitution is therefore not observed. It remains to be determined whether naive CD4 T cell reconstitution is indeed hampered in individuals who have low CD4 T cell counts at the start of therapy or whether this group will eventually reach naive CD4 T cell levels similar to those in the group with higher baselines in a few more years.

Strikingly, the proliferation marker Ki67 was still slightly elevated in adults after 7 years of HAART, even though CD4 T cell counts had normalized. Although we found no correlation between T cell activation markers and the fraction of Ki67+ T cells, residual viral activity, which for the most part does not exceed the detection threshold, may be responsible for the observed proliferation levels in the various T cell subsets.

Taken together, these data demonstrate an unanticipated capability of the immune system to eventually recover from severe T cell depletion, provided that HIV viremia is properly controlled. HIV-infected children were able to reconstitute the T cell compartment well at any stage of lymphodepletion, whereas adults with low baseline CD4 T cell counts took longer to normalize CD4 T cell counts, and did not have normalized naive T cell fractions after long-term HAART. On a phenotypical basis, in children, but unexpectedly also in adults with more than 200 CD4 T cells/μl blood at start of therapy, naive T cells were generated such that reconstitution of the immune system did not lead to accelerated aging of the T cell compartment.

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Disclosures

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References


