

Early recovery of CD4+ T lymphocytes in children on highly active antiretroviral therapy

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Introduction: Regeneration of CD4+ T lymphocytes has been shown to be thymus-dependent in bone marrow transplant recipients and after intensive chemotherapy. The rate of CD4+ T cell regeneration is correlated positively with enlargement of the thymus, as shown on radiographs, and higher rates of CD4+ T lymphocyte regeneration were observed in children as compared with adults, consistent with thymic function diminishing with age. We hypothesized that in HIV infected patients CD4+ T cell recovery during highly active antiretroviral therapy (HAART) may also be thymus dependent. Therefore, repopulation of naive (CD45RA+), memory (CD45RO+) and total CD4+ T lymphocytes and total CD8+ T lymphocytes in peripheral blood was assessed in 13 HIV infected children during the initial 3 months of HAART.

Results: Significantly higher recovery rates of naive, memory and total CD4+ T cells were observed in children below the age of 3 years as compared with older children. Kinetics of total CD8+ T cells showed no relation to age. Moreover, recovery rates of naive CD4+ T cells in patients below 3 years of age were 10–40 fold higher as compared with previously reported naive CD4+ T cell recovery rates in adults on HAART.

Conclusions: High recovery rates of naive, memory and total CD4+ T cells can be achieved in children below 3 years of age. Changes in CD8 counts did not correlate with age. These results indicate that regeneration of CD4+ T cells during HAART may be a thymus-dependent process.

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Introduction

Treatment of HIV infected adults with highly active antiretroviral treatment (HAART) results in a decline in plasma HIV RNA levels and an increase in circulating CD4+ T cells. However, because of slow CD4+ T cell regeneration in adults, complete restoration of CD4+ T cell numbers and function, if possible at all, will take considerable time. The reconstitution pattern of CD4+ T cells in peripheral blood during HAART is biphasic: an initial rapid rise in CD4+ T cells in the first month of treatment is followed by a much lower rate of CD4+ T cell increase. The initial rapid CD4 cell rise is predominantly due to an increase in circulating memory CD4+ T cells. Recovery rates of naive CD4+ T cells are low throughout the course of treatment [1,2].

In humans, the capacity to regenerate CD4+ T cells declines with age. In children who received antineoplastic chemotherapy or bone marrow transplantation, the rate of CD4+ T cell regeneration was higher as compared with adults because of production of CD45RA+ (naive) CD4 cells [3,4,5]. In these children, increased production of CD45RA+/CD4+ T cells is associated with thymic enlargement as shown on radiographs. On the other hand, in adults, sustained depletion of CD4+ T cells has been observed after intensive antineoplastic chemotherapies, bone marrow transplantation (BMT) or CD4 depletion therapy for multiple sclerosis or rheumatoid arthritis using anti CD4 antibodies [3-9]. The difference in CD4+ T cell recovery rates between children and adults suggests that the pathway of CD4+ T cell regeneration is thymus dependent [10] and that the capacity for CD4+ T cell regeneration diminishes with age as a result of thymic involution.

In contrast, thymus independent pathways exist for regeneration of CD8+ T cells, via peripheral expansion of mature CD8+ T cells in the lymphoid organs [11,12]. The thymus-dependence of CD4+ T cell regeneration leads one to expect a more pronounced increase of CD4+ T cells following onset of potent antiretroviral therapy in children as compared with HIV infected adults. In this study we assessed repopulation of CD4+ and CD8+ T cells and naive and memory CD4+ T cells in the blood of HIV infected children on HAART.

Methods

Plasma HIV RNA levels and kinetics of CD4+ and CD8+ T cell subsets were analysed as part of a study investigating the efficacy and safety of combination therapy with zidovudine, lamivudine and indinavir in

HIV infected pediatric patients. Dosages of each drug were as follows; zidovudine: 360 mg/mm² (body surface; three times a day); lamivudine: > 30 kg: 150 mg (twice a day), < 30 kg: 8 mg/kg body weight/day (twice a day); Indinavir 100-200 mg/day/kg metabolic weight (three times a day). Blood samples were obtained at baseline and after 2 weeks, 4 weeks, 3 months and 6 months from the onset of therapy. Compliance regarding medication intake was assessed during each visit.

Plasma HIV RNA levels were measured using a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay (Amplicor HIV-1 Monitor, Roche Diagnostic Systems, Branchburg, New Jersey, USA). The lower limit of detection was 400 copies/ml. Lymphocyte immuno-phenotyping for CD4+ and CD8+ T cells was determined in whole blood by two colour flow cytometry, using anti CD3, anti CD4, anti CD8, anti CD45RA and anti CD45RO monoclonal antibodies. CD4+ T lymphocytes expressing the CD45RA surface antigen were considered to be thymic emigrants and naive cells, CD4+ T lymphocytes expressing the CD45RO surface antigen were considered to be memory type cells.

In the analysis, data were included from patients who were at least 90 days on therapy and of whom immuno-phenotyping data were available from at least three time-points: at baseline; one at 90 days; and at least one in between (at week 2 and/or week 4 after initiation of therapy). Data from patients with reported non compliance regarding intake of medication, were excluded from analysis.

At the start of this analysis, 26 patients had entered the cohort. Data from 13 children could not be used for analysis because of the following reasons: for eight children (0.95-14.9 years old) no blood samples from week 12 were available for immuno-phenotyping and for two other children (3.1 and 7.3 years old) no blood samples were available from baseline. Two patients (1.1 and 2.5 years old) were non compliant regarding intake of medication. For one patient (7.3 years old),

Table 1. Baseline characteristics.

Patient	Age (years)	Plasma HIV RNA (copies/ml)	CD4 (cells/ μ l)
a	0.80	682000	1720
b	0.88	761500	1154
c	2.88	51220	153
d	5.39	145273	195
e	5.40	194300	192
f	6.99	32580	4
g	7.70	65700	65
h	9.62	16800	284
i	10.55	18400	202
j	11.17	201700	4
k	13.16	393420	48
l	13.23	1345	233
m	16.33	1910	11

no blood samples at all were collected for immunophenotyping. The median age of the group excluded from analysis was 3.7 years (range 0.95–14.9 years), whereas the median age of the included group was 7.7 years (range 0.8–16.3 years). Median CD4 count at baseline of the excluded group was: 502 cells/ μ l (range 293–1864 cells/ μ l). In the included group the median CD4 baseline count was 202 cells/ μ l (range 4–1720 cells/ μ l) (Table 1).

Results

Thirteen children were included in the analysis of whom the baseline characteristics are shown in Table 1. Median plasma HIV RNA level at baseline was 69625 copies/ml. During the initial 3 months of therapy median HIV RNA levels decreased significantly to 500 copies/ml. Median total CD4+ T cell levels rose from 202 (range 4–1864) cells/ μ l to 327 (range 11–2150) cells/ μ l. Median total CD8+ T cell levels decreased from 936 to 880 cells/ μ l. The responses of naive and memory and total CD4+ T cells of two representative patients (b and e) are shown in Fig. 1. Linear regression analysis was used to calculate recovery

rates in blood of total CD4 and CD8 cells and memory and naive CD4 cells during the first 90 days of treatment. Recovery rates were expressed as the change in the absolute number of cells per μ l blood per day, and are shown in Table 2.

Recovery rates of total CD4+ and total CD8+ T cells and memory and naive CD4 cells were plotted as a function of age at the start of therapy (Fig. 2). A significant difference in recovery rates of naive, memory and total CD4+ cells was observed between children younger than 3 years of age and older children ($P < 0.02$, Wald-Wolfowitz test) (Fig. 2a,b,c). None of the patients above the age of 3 years had CD4+ T cell recovery rates (total, naive or memory) higher than any of the patients below 3 years of age. In contrast, recovery rates of CD8+ T cells did not show any relation to the age of the children (Fig. 2d).

Discussion

We observed that recovery rates of naive (CD45RA+), memory (CD45RO+) and total CD4+ T cells were higher in patients under 3 years as compared with older children. In three children below 3 years we observed

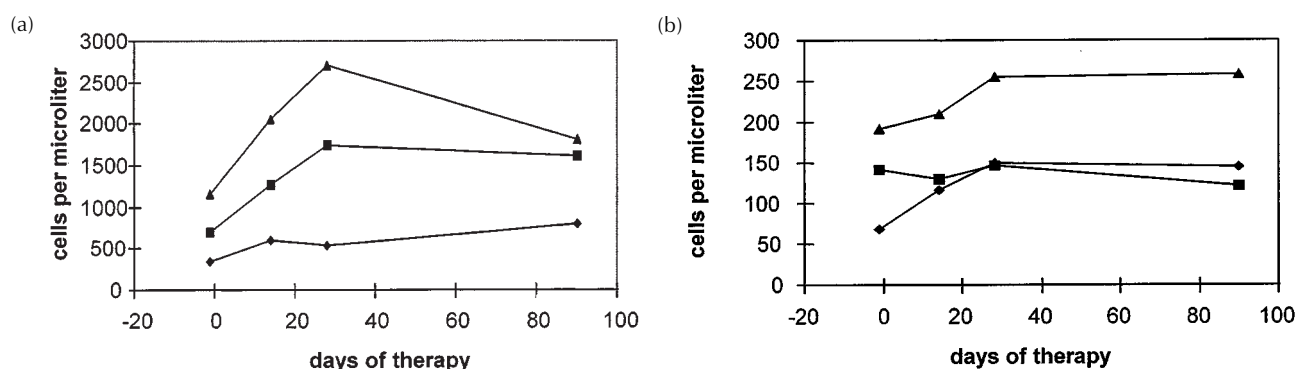


Fig. 1. Response of naïve, memory and total CD4+ T lymphocytes in peripheral blood of patient b (a) and patient e (b). \blacklozenge CD4+/CD45RO+; \blacksquare CD4+/CD45RA+; \bullet Total CD4+.

Table 2. Rates of lymphocyte recovery and viral load reduction during the initial 3 months of therapy.

Patient	Age (years)	Recovery rate (cells/ μ l/day)		Recovery rate (cells/ μ l/day) Total CD4+	Recovery rate (cells/ μ l/day) Total CD8+	Viral load reduction (10 log)
		CD4+/CD45RA+	CD4+/CD45RO+			
a	0.80	9.30	7.13	16.4	0.17	2.99
b	0.88	8.58	4.10	12.70	16.20	3.18
c	2.88	3.20	1.90	5.80	3.20	2.11
d	5.39	-0.33	-0.45	-0.78	-1.40	2.56
e	5.40	-0.18	0.64	0.47	-6.75	2.59
f	6.99	0.44	1.11	1.54	18.40	1.06
g	7.70	0.57	-0.21	0.36	1.16	1.79
h	9.62	1.00	0.42	1.42	-1.52	1.53
i	10.55	0.65	0.05	0.69	-0.02	1.57
j	11.17	-0.02	0.02	0.004	7.28	0.12
k	13.16	1.29	0.52	1.81	1.82	3.11
l	13.23	0.30	0.08	0.38	-0.80	0.43
m	16.33	0.01	0.08	0.09	-0.98	0.58

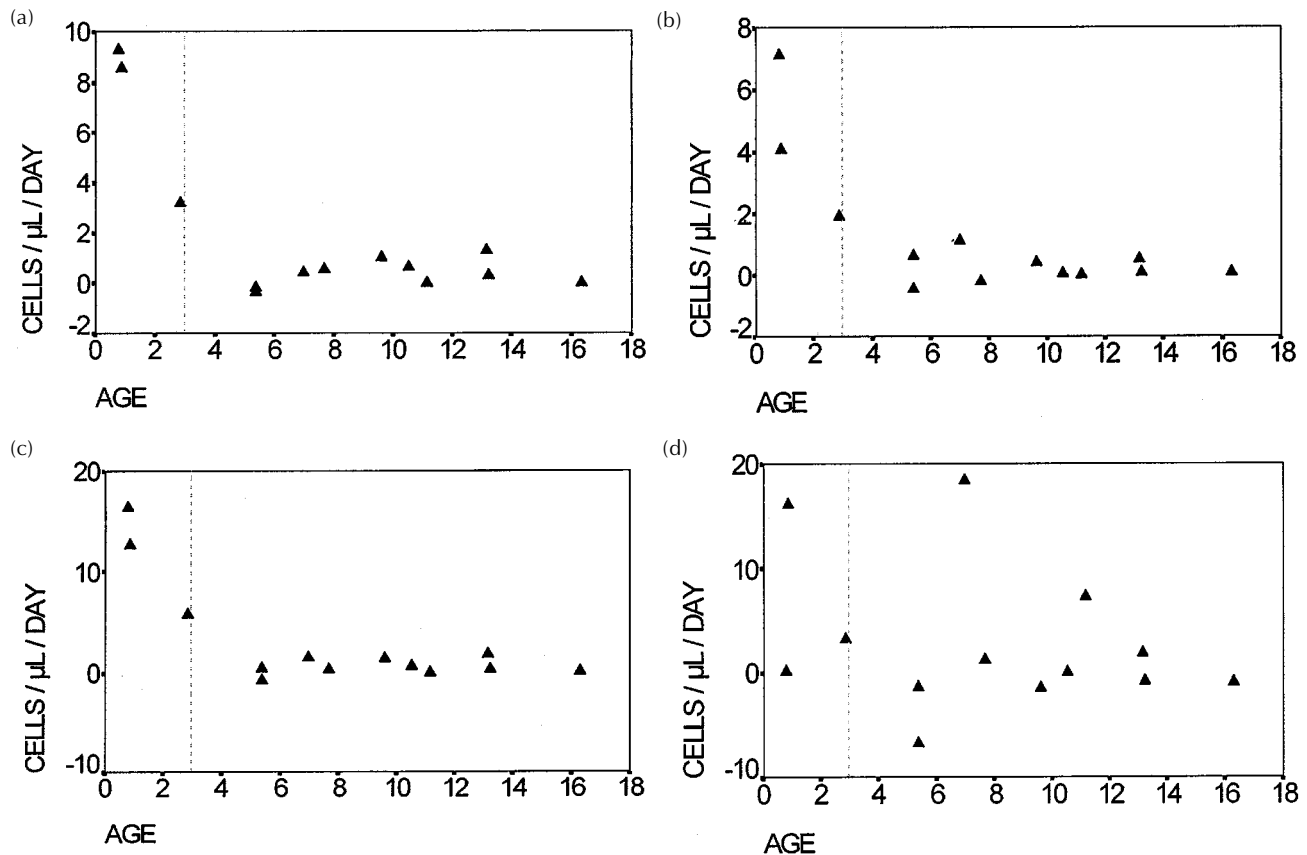


Fig. 2. Recovery rates of (a) CD4⁺/CD45RA⁺ (b) CD4⁺/CD45RO⁺ (c) CD4⁺ and CD8⁺ T lymphocytes (cells/ μ l/day) versus age (years).

recovery rates of naive CD4⁺ T cells of 3.20, 8.58 and 9.30 cells/ μ l/day, which is 10–40 fold higher as compared with recovery rates of naive CD4⁺ T cells in adults (0.2–0.4 cells/ μ l/day) [1] (Cohen Stuart *et al.*, unpublished data). Although the number of patients in this analysis is limited, our data clearly demonstrates the possibility of rapid recovery of naive CD4⁺ T cells in young infants below 3 years of age.

These observations may be explained in several ways. Because the patients in this study were horizontally infected, except for patient h, k and m (9.6, 13.2 and 16.3 years, respectively) the age of the patient is generally equal to the duration of HIV infection. Therefore, the shorter duration of HIV infection in the youngest children may explain the high CD4⁺ T cell recovery rates. An age dependent capacity to redistribute T lymphocytes from lymphoid tissues into the circulation after the start of HAART could also account for differences in CD4 cell recovery rates. However, such an explanation is inconsistent with the absence of any relation between age and CD8 cell kinetics during HAART in this set of children.

When comparing the three youngest patients (a–c) with the others (d–m), it should be noted that patients

a and b had higher baseline CD4 counts in comparison with the others. This is probably related to their age. Babies often have high numbers of circulating lymphocytes. It may be argued that high CD4⁺ T cell recovery rates in these young children (less than 3 years) may result from high CD4⁺ T cell baseline values (Table 1). Secondly, patient a and b had higher plasma viral loads at baseline in comparison with the other patients. However, in adults, it has been demonstrated that CD4 recovery does not depend on either baseline plasma HIV RNA or baseline CD4 count values [13]. Furthermore, no significant difference in the magnitude of viral load reduction was found between the group of youngest (a–c) and the group of older patients (d–m) (Wald-Wolfowitz test: $P = 0.71$).

Theoretically, CD45 isotype switching from CD45RO to CD45RA phenotype may also account for an increase in CD45RA⁺/CD4⁺ T lymphocytes [15–17]. However, direct evidence of reversion from memory to naive phenotype in CD4 T lymphocytes has exclusively been found in experimental murine models. Data on reversion of memory to naive phenotype are conflicting [12] and it is unclear to what extent this phenomenon plays a role in humans *in vivo*. Moreover, a rapid increase of CD45RA⁺ CD4⁺ T cells occurred

in patients who also had a rapid increase of total CD4+ T cells. Therefore, if thymus input of CD45RA+ cells plays a minor role and peripheral CD4 T cell expansion accounts for CD4 T cell increase, the reversion from CD45RO to CD45RA would have to occur at a substantial level in dividing CD4 cells. This is inconsistent with data demonstrating CD45RO expression in dividing T cell populations [18].

We conclude that high recovery rates of naive CD45RA+/CD4+ T cells in patients less than 3 years of age during HAART may be best explained by the presence of a functional thymus. Recently, it was reported that regeneration of CD45RA+/CD4+ T cells and expansion of the volume of the thymus are positively correlated in HIV infected children during HAART [14]. After antineoplastic chemotherapy, the rate of CD4 cell recovery gradually diminishes with age [3,4]. In children on HAART, we did not find a gradual decrease in the rate of CD4 recovery but in subjects older than 3 years we observed CD4 recovery rates comparable to the rates of adult HIV infected patients on HAART. This may be explained by precocious involution of the thymus, as a result of HIV mediated destruction of thymic parenchyma.

In summary, we demonstrated that high recovery rates of naive, memory and total CD4+ T cells can be achieved in children below the age of 3 years on HAART, whereas CD8+ T cell kinetics showed no relation to age. This indicates that regeneration of CD4+ T cells during HAART uses a thymus-dependent pathway.

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