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Restoration of recent thymic emigrant CD4$^+$ T cells is associated with sustained adherence to antiretroviral treatment in HIV-infected children

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Abbreviations: ART: antiretroviral treatment, RTE: recent thymic emigrant CD4$^+$ T cells.

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Abstract

To evaluate the levels of recent thymic emigrant (RTE) $CD4^+$ T cells in HIV-infected children and to explore the associations among their frequency, antiretroviral treatment (ART) adherence, and $CD4^+$ T cell restoration. The group evaluated comprised 85 HIV-infected patients classified as subjects with moderate or severe immunosuppression or as those with no evidence of immunosuppression. To evaluate the association between the frequency of RTE $CD4^+$ T cells and ART adherence, 23 of the 85 patients were evaluated at two different time points during a one-year follow-up period. Children with severe immunosuppression had lower frequencies of RTE $CD4^+$ T cells compared with children without evidence of immunosuppression ($P<0.001$). The frequency of RTE $CD4^+$ T cells in children with a high rate of adherence was significantly higher ($P<0.05$) than that observed among those with suboptimal adherence. The latter group presented with infectious intercurrences on admission that decreased after initiation of treatment along with improved $CD4^+$ and RTE naïve $CD4^+$ T cells counts. The adequate ART adherence is essential for immune reconstitution, which might be reflected by the levels of RTE $CD4^+$ T cells.

Key words: Paediatric HIV, recent thymic emigrants, treatment adherence.

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Introduction

The gradual depletion of $CD4^+$ T cells and chronic immune activation are central aspects of human immunodeficiency virus (HIV) pathogenesis and are associated with alterations in the number and phenotype of $CD4^+$ T cell subsets [1-2]. In the paediatric population, the success of

antiretroviral treatment (ART) is directly associated with correct adherence [3-5]. Immune reconstitution after therapy, particularly in children, involves naïve $CD4^+$ T cells due to their more effective production of these cells in this developmental period; however, in adults, the reconstitution of the naïve $CD4^+$ T cell pool is achieved by the expansion of existing peripheral naïve T cells [6-8].

CD31, also known as platelet cell adhesion molecule 1 (PECAM 1), is a 130 kDa surface glycoprotein that belongs to the immunoglobulin superfamily. This molecule is involved in the migration of cells through the endothelium cell junction and mediates cell-to-cell adhesion. CD31 is expressed on a variety of cell types, including thymocytes, lymphocytes, endothelial cells, circulating monocytes, and granulocytes.

Because CD31 is expressed on the majority of thymic cells and in 85% percent of $CD4^+$ T cells from cord blood, this molecule was selected as a useful marker of recent thymic emigrants (RTE). In addition, the presence of CD31 typifies naïve $CD4^+$ T lymphocytes that have not undergone significant homeostatic proliferation after their exit from the thymus [9-14]. In previous studies, in order to identify additional markers for monitoring treatment and disease progression in children with HIV/AIDS, we have shown variations in naïve and other $CD4^+$ T cell subsets levels [15-16].

The aim of the present study was to evaluate, through the expression of CD31, the levels of RTE $CD4^+$ T cells in children with different degrees of immunosuppression. In addition, we explored the associations among the frequency of RTE, adherence to ART and the restoration of $CD4^+$ T cells.

Materials and Methods

Study population

The group of children and adolescents evaluated comprised 84 mother-to-child HIV-infected patients (40 boys and 44 girls, aged between 18 months and 13 years) and 1 male adolescent with horizontal infection treated at the authors' College Hospital. HIV infection was confirmed by enzyme-linked immunosorbent assay (ELISA), western blot analysis and virologic assays. Patients were selected on the basis of their clinical and immunological status according to the Centers for Disease Control and Prevention (CDC) 1994 Paediatric Classification [17]. HIV-infected children

were divided into two groups (Table 1): Group A included subjects who were moderately or severely immunosuppressed (i.e., $CD4^+$ T cells < 24% of total lymphocytes, $n = 40$), and Group B included subjects with no evidence of immunosuppression (i.e., $CD4^+$ T cells ≥ 25% of total lymphocytes, $n = 45$). To evaluate the association between the frequency of RTE $CD4^+$ T cells and adherence to ART, 23 of the 85 HIV-infected children were evaluated at two different time points during an average one-year follow-up period. Control samples were obtained from 10 HIV-seronegative healthy children, aged between 22 months and 14 years, among children coming to the hospital as controls for studies of programmed traumatology surgeries. All patients were evaluated clinically and for their HIV RNA levels.

Antiretroviral treatment included nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Adherence was monitored through surveys and the counting of administered medication. The criteria provided by the World Health Organization (WHO) were used as a guide to estimate adherence to treatment. Adequate compliance was considered when the adherence to treatment was equal to or greater than 95% [3]. Written informed consent was obtained from the parents of all participants. The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Institution Ethical Committee (registration number 599/15).

RTE $CD4^+$ T cell phenotyping

Phenotypic analysis was performed on whole blood by using the following monoclonal antibodies: CD4 and CD8 [peridinin chlorophyll (PerCP)], CD45 and CD45RA [fluorescein isothiocyanate (FITC)], CD14, CD31 and CD62L [phycoerythrin (PE)], all from BD Biosciences (San Jose, CA, USA). The isotype antibodies were used as negative staining controls. The viability of the cells was evaluated by trypan blue staining, and the viability range was observed to be higher than 90%. The data were acquired on a FACS Calibur cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and analysed with CellQuest software (Becton Dickinson). $CD4^+$ T cells were defined as naïve ($CD4^+CD45RA^+CD62L^+$), RTE ($CD4^+CD45RA^+CD31^+$), central memory ($CD4^+CD45RA^-CD62L^+$), effector memory ($CD4^+CD45RA^-CD62L^-$) and terminally differentiated ($CD4^+CD45RA^+CD62L^-$).

Plasma HIV RNA viral load

The HIV RNA level was determined using Nuclisens EasyQ HIV-1 version 2 (bioMérieux, Marcy l'Etoile, France). The results are expressed as $\log_{10}$ of the number of IU/ml, with a detection threshold of 50 IU/ml (i.e., log <1.70).

Statistical analysis

The normality of the variable distribution was assessed by using the Kolmogorov-Smirnov criterion. Analysis of variance (ANOVA) and the Student-Newman-Keuls test were applied to compare the levels of $CD45RA^+CD31^+CD4^+$ T cells among the different clinical groups evaluated. Student's t test or the Mann-Whitney test of paired samples was used as appropriate to compare the total RTE $CD4^+$ T cells and HIV viral load at the two time points of the longitudinal follow-up. Proportions were compared by Fisher's exact test. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using the GraphPad Prism 5.0 software.

Results

Immune and virological characteristics of the study participants

The main immune and virological characteristics of the entire study population are summarized in Table 1. All except one of the 85 paediatric patients acquired HIV infection by vertical transmission, but no prophylactic measures were applied in the HIV-infected mothers, neither during pregnancy nor during delivery. The ages of diagnosis in the children with moderate or severe immunosuppression (i.e., Group A) were similar to those without evidence of immunosuppression (i.e., Group B) (range = 1.4–15 and 3–14 years, respectively). The children with no evidence of immunosuppression had a longer time on ART associated with higher levels of $CD4^+$ nadir T cells and a higher proportion of children with a viral load below the level of detection compared with the children with moderate or severe immunosuppression (Table 1). The children in Group A had diminished $CD4^+$ T cell counts whereas naïve $CD4^+$ T cells were diminished both as percentages and counts. In contrast, the percentages of central and effector memory $CD4^+$ T cells were higher, and $CD4^+$ T cell counts of these subsets were lower, in Group A compared with Group B (Table 1). Terminally differentiated $CD4^+$ T cell counts were also decreased in Group A. As expected, $CD8^+$ T cell percentages and counts were increased in children with severe immunosuppression (Table 1).

A group of 23 patients was also evaluated at two different time-points, with an average follow-up period of 12 months (range, 6–15 months). Seven of these patients were already receiving the appropriate ART, with correct adherence, while 8 patients presented with virological failure due to deficient adherence to treatment. Of the remaining 8 patients, HIV infection had recently been diagnosed in 3 children, and thus, they were naïve of treatment at inclusion, while the other 5 children restarted ART after a variable period of abandonment.

Decreased number of RTE CD4$^+$ T cells in the peripheral blood of HIV-infected children

The frequency of recent thymic emigrant CD4$^+$CD45RA$^+$CD31$^+$ T cells was quantified in 85 HIV-infected children and adolescents. A diagram of the flow cytometry gating strategy and representative dot-plots of the RTE CD4$^+$ T cells in the HIV-infected children with moderate or no signs of immunosuppression and uninfected controls are shown in Figure 1. Lower percentages of CD4$^+$CD45RA$^+$CD31$^+$ T cells were recorded in the HIV-infected children compared with the uninfected controls. The children with moderate or severe immunosuppression also had lower percentages (Figure 2A) and absolute counts (Group A, median= 106 cells/μL), range= 60–181; Group B, median= 473 cells/μL), range= 334–652) of CD4$^+$CD45RA$^+$CD31$^+$ T cells compared with the children without evidence of immunosuppression.

Monitoring of RTE CD4$^+$ T cells in HIV-infected children under retroviral treatment.

The decreased percentage of RTE CD4$^+$ T cells found in the subjects with moderate or severe immunosuppression prompted us to investigate whether changes in the levels of this T subset were linked to the efficacy of and adequate adherence to antiretroviral treatment. To address this issue, CD4$^+$CD45RA$^+$CD31$^+$ T cells were prospectively measured in 23 HIV-infected children during a 12-month follow-up period. According to the time of implementation of treatment and the rate of treatment adherence, the patients were divided into 3 groups: AD1 comprised patients who were already receiving the appropriate ART with a high adherence rate, and AD2 comprised treatment-naïve patients who had been recently diagnosed with HIV infection and started treatment after recruitment, as well as patients who had discontinued ART and reinitiated treatment after recruitment. Both subgroups of patients included in the AD2 group had a high adherence rate during the follow-up period. The third group comprised a non-adherent group (NA) of patients

receiving ART but with detectable levels of viral load due to deficient treatment adherence. Despite changes in the ART schedule according to the results of a genotyping test, a low adherence rate was maintained during follow-up.

In the children of the AD1 group, who maintained a high number of $CD4^+$ T cells and an undetectable viral load, RTE $CD4^+CD45RA^+CD31^+$ T cells did not show significant changes during follow-up (Table 2, Figure 2B). However, in 4 out of 7 patients, the frequencies at 12 months of follow-up were in the range of those observed in uninfected controls (Figure 2B). In the AD2 patients, a significant increase in $CD4^+$ T cells and a decrease in viral load were accompanied by a significant increase in RTE and total naïve $CD4^+$ T cells following 12 months of follow-up (Table 2, Figure 2B). In contrast, the group of non-adherent patients (NA) presented decreased levels of $CD4^+$ T cells, high viral loads and decreased values of RTE and total naïve $CD4^+$ T cells, which were all maintained during follow-up (Table 2, Figure 2B). Of note, the frequency of RTE $CD4^+$ T cells in the children with a high rate of adherence (i.e., groups AD1 and AD2) were significantly higher than those observed in the children with a low rate of adherence (i.e., NA) regardless of the time point assessed (Figure 2B).

Association between infectious intercurrence and ART adherence rate in HIV-infected children.

We then assessed the extent to which the rate of treatment adherence and virological response were associated with the presence of infectious intercurrences during the follow-up period. None of the children in the AD1 group presented with relevant infectious pathology during the follow-up period. In contrast, 75% of the patients in the AD2 group presented with some kind of infectious intercurrence on admission. Of note, two adolescents included in the AD2 group showed intercurrences at admission. One of these patients was the only patient at inclusion with a recent diagnosis of HIV for sexual transmission, co-infected with acquired syphilis, while the second patient, infected by mother-to-child transmission, was diagnosed late, at twelve years of age, and had pneumonia. After the initiation of treatment and with the achievement of an appropriate immune and virological response, the AD2 patients were free from infectious processes at the end of the study period. Two patients of the AD2 group and one non-adherent patient presented with two intercurrences simultaneously. Pneumonia-herpes zoster infection and

pneumonia-oral candidiasis were the simultaneous intercurrences in the patients of the AD2 group at the initiation of the study, whereas *Microsporidium* spp. diarrhoea-oral candidiasis was observed in a non-adherent patient. Moreover, oral candidiasis was recurrent in one patient in the NA group, while three other children in this group presented with and maintained different infectious comorbidities, such as recurrent herpes zoster infection or relapse of pulmonary infection due to *Mycobacterium tuberculosis*. It is worth noting the decrease in the number of infectious conditions, especially in the lungs and mouth, in the eight patients in the AD2 group after initiation of treatment (Table 3).

Discussion

The number and functional characteristics of the different lymphocyte subsets present alterations during the course of HIV infection. Progressive immune exhaustion is a much more complex process than a simple reduction in the number of $CD4^+$ T lymphocytes, with striking differences observed in the natural course of the infection between children and adults [18]. In adults, circulating viral levels reach their peak during the acute infection phase and decrease after 6 or 8 weeks, and occasionally, the viral load can be maintained at undetectable levels for years. In contrast, in children without therapy, the viral load reaches very high levels during the first two years of life and does not decrease as quickly as in adults [19].

In this observational study, we evaluated whether RTE cells varied not only between the immunological categories but also with the degree of adherence in HIV-infected children. We showed that the children with moderate or severe immunosuppression had the lowest levels of RTE and that a correct adherence promoted the restoration of thymic function and decreased the presence of comorbidities and intercurrences in HIV-infected children.

The children with severe immunosuppression had lower levels of naïve and RTE
$CD4^+$ T cells compared with the children with no evidence of immunosuppression. These findings are probably due to an enhanced need for the recruitment of RTE to regenerate mature naïve T cells in patients with major homeostatic disturbances compared with those in more stable conditions in which cell loss and cell production appeared more balanced [20, 21]. The children with no evidence of immunosuppression also showed higher central and effector memory $CD4^+$ T cell

counts but lower percentage levels of these subsets compared with the children with severe immunosuppression. This discrepancy might be accounted for by the fact that children with better clinical conditions had higher total $CD4^+$ T cell counts.

Protracted basal activation of the immune system in response to HIV infection, which cannot be completely neutralized by the action of ART, could be related to the incomplete normalization of RTE $CD4^+$ T cells observed in this study in children without evidence of immunosuppression. An inverse correlation between the levels of RTEs and activated $CD8^+$ T cells was observed in HIV-infected adolescent [22] and adult patients [23]. Altered thymic function might be secondary to HIV infection due to the production of inhibitory cytokines and an increased conversion of naïve cells into memory T cells [9]. Of note, in the children with correct treatment adherence, the number of RTE $CD4^+$ T cells increased along with the number of total naïve $CD4^+$ T cells.

The longitudinal monitoring of RTEs allowed us to relate the response to therapy and the conditions of adherence to ART with the values of RTEs. The children with sustained adherence had the highest levels of RTEs, which remained stable during the follow-up period, contrasting with the constantly low RTE cells in the non-adherent children. These findings support the hypothesis that only sustained control of the viral load can restore thymic function. However, the introduction of ART followed by correct adherence was able to significantly improve RTEs levels during follow-up although not to the levels of the patients who had sustained adherence over more than eight years or to the levels found in uninfected children. This finding indicates that a prolonged and continuous high level of adherence would probably be necessary to permit a complete reconstitution of the immune system, although a significant reduction in the number of patients who presented with intercurrences was observed during the time of follow-up in the children with a better rate of adherence. Moreover, the two adolescents who had good adherence did not recover RTEs at the same rate as younger children (data not shown), supporting the hypothesis that a highly active thymus in early childhood may contribute to better immune reconstitution if ART is initiated early in life. In line with our findings, adult HIV/HCV co-infected patients show lower levels of RTEs than single HIV-infected subjects [24].

RTE T cells have also been identified by molecular biology techniques by analysing the excision circles of the T cell receptor (TRECs) [25, 26]. Positive correlations between the content of TRECs

and the frequency of RTE naïve $CD4^+$ T cells have been described in different pathologies [27-29], confirming the use of CD31 as a marker of thymic production.

One limitation of the study is that the follow up evaluation of RTE in patients with different rates of adherence was performed in only a small patient group. Functional reconstitution is the fundamental objective of ART, since it allows the immune system to perform its key functions, such as protection against opportunistic microorganisms. Achieving the restoration of immune function with adequate competence to control successive infectious challenges will result in the best quality of life of HIV-infected children. This last goal can only be achieved through the commitment of the parents and guardians of this vulnerable population to the responsible administration of ART.

Authors' contributions.

Conceived and designed the experiments: EG, JB; performed the experiments: AU, JB; recruitment and clinical monitoring MC, GB; analysed the data: SL, EG, JB, GB; wrote the paper: GB, SL, EG.

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Table 1. Clinical and immunological characteristics of the full study population

Variables	Group A (n= 40)	Group B (n= 45)	Co (n= 10)	P value
Sex (males/total)	19/40	22/45	5/10	
Age [A] (years)	11.2 (1.4-15)	9.3 (3-14)	10.5 (1.8-14)	
Time on ART [A] (years)	3.74 (0-11)	7.97 (1-11)	ND	A vs B <0.05
Time untreated [A] (years)	5.91 (1.5-11)	2.75 (1.5-9)	ND	A vs B < 0.05
HIV RNA [A] (Log 10 copies/mL)	3.24 (1.81-4.67)	1.91 (<1.70-2.53)	ND	A vs B < 0.05
No. of children with VL Log 10 < 1.70 (%)	8 (20)	35 (78)	ND	A vs B < 0.001
Lymphocyte count [A] (cells/µL)	2905 (1072-4702)	3101 (1023-4093)	2700 (2100-3300)	
$CD4^+$ T cells [A] (%)	11 (8-18)	37 (31-43)	47 (40-56)	A vs B, A vs Co < 0.05
$CD4^+$ T cell count [A] (cells/µL)	423 (72-762)	978 (692-1500)	1296 (1080-1512)	A vs B, A vs Co < 0.05
$CD4^+$ T cells nadir [A] (%)	9 (3-15)	20 (6-36)	ND	A vs B < 0.05
Naïve $CD4^+$ T cells [A] (%)	36 (31-43)	50 (38-64)	55 (49-62)	A vs B, A vs Co < 0.05
Naïve $CD4^+$ T cell count [A] (cells/µL)	170 (5-338)	598 (299-913)	712 (582-842)	A vs B, A vs Co < 0.05
Central memory $CD4^+$ T cells (%)	45 (33-59)	34 (22-56)	31 (24-36)	A vs B, A vs Co < 0.05 B vs Co > 0.05
Central memory $CD4^+$ T cell count (cells/µL)	191 (147-239)	369 (325-421)	400 (387-417)	A vs B, A vs Co < 0.05
Effector memory $CD4^+$ T cells (%)	12 (15-19)	9 (5-14)	3 (1-5)	A vs B, A vs Co < 0.05
Effector memory $CD4^+$ T cell count (cells/µL)	46 (26-72)	100 (83-115)	39 (36-42)	A vs B, B vs Co < 0.05
Terminally differentiated $CD4^+$ T cells (%)	3 (1-5)	3 (1-5)	2 (1-3)	
Terminally differentiated $CD4^+$ T cell count (cells/µL)	10 (13-17)	24 (16-33)	26 (13-39)	A vs B, A vs Co < 0.05
$CD8^+$ T cells (%)	57 (45-73)	35 (26-47)	23 (15-28)	A vs B, A vs Co < 0.05

$CD8^+$ T cells count (cells/μL)	1498 (707-2325)	983 (482-1474)	939 (777-1101)	A vs B, A vs Co < 0.05

Note. [A] Data are given as median and range; ND, not done. Group A, subjects with moderate or severe immunosuppressio (i.e., $CD4^+$ T cells < 24%), Group B, subjects with no evidence of immunosuppression (i.e., $CD4^+$ T cells ≥ 25%). Co, uninfected controls. Comparisons among groups were performed by ANOVA.

Table 2. Immune and virological characteristics of 23 HIV-infected children with different rates of ART adherence

Variables [A]	AD1 (n= 7)		AD2 (n= 8)		NA (n= 8)	
	t0	t1	t0	t1	t0	t1
CD4 (%)	33 (18-48)	33(20-46)	16 (4-26) [B]	29(19-39)	16(4- 19)	19(7-21
$CD4^+$ T cell count (cells/µL)	1461(1045-1877)	1237 (786-1688)	268 (38-614) [B]	749 (200-1338)	314 (177-541)	392 (175-609)
Log VL	< 1.70	<1.70	4.9 (2.84-6.34) [B]	<1.70	3.90 (2.4-4.87)	3.36 (2.56-3.99)
Naïve $CD4^+$ T cells (%)	54(43-66)	62 (51-80)	39 (24-58) [B]	55 (41-69)	34 (11-49)	32 (19-60)
Naïve $CD4^+$ T cell count (cells/µL)	719 (492-1106)	787 (308-1346)	90 (13-215) [B]	397 (154-840)	121 (20-211)	157 (28-311)
$CD4^+CD45RA^+CD31^+$ (%) (Ratio t1/t0)	1.27 (1-2.33)		2.63 (1.75-7) [C]		1.34 (0.33-1.60)	
$CD4^+CD45RA^+CD31^+$ T cell count (cells/ µL)	0.90 (0.57-1.10)		5.26 (2.87-17.6) [C]		1.44 (0.21-2.68)	

(Ratio t1/t0)

Notes. [A] Data are given as median and range. AD1, patients receiving ART with good adherence; AD2, treatment-naïve patients recently diagnosed with HIV infection, or patients with discontinued ART that restarted treatment; NA, patients with a low adherence rate. [B] $P < 0.05$ vs. t1 by paired comparison; [C] $P < 0.05$ vs. AD1 and NA by ANOVA.

Table 3. Infectious intercurrences in 23 HIV-infected children with different rates of ART adherence.

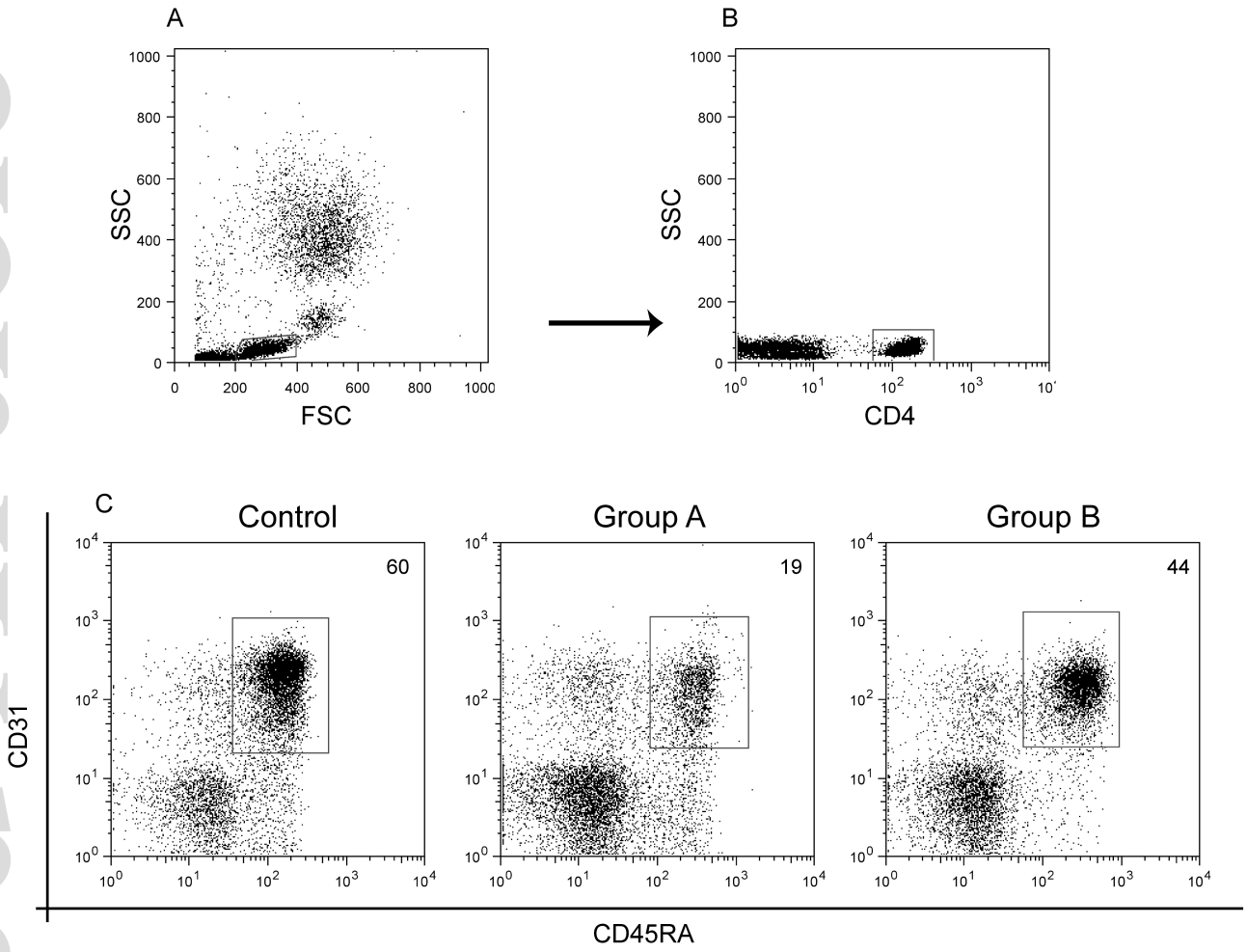
	AD1		AD2		NA	
Patient groups [A] (n)	7		8		8	
Age at inclusion Median years (range)	10.8 (4-17)		9.27 (0.2-15)		12.5 (5-17)	
Years of ART at inclusion Median (range)	8.1 (4-17)		0		9.2 (3-16)	
No. of comorbidities [B]	t0	t1	t0	t1	t0	t1
Oral candidiasis	0	0	2	0	2	1
Pneumonia	0	0	3	0	0	0
Ocular toxoplasmosis	0	0	1	0	0	0
Herpes zoster	0	0	1	0	1	1
Pulmonary tuberculosis	0	0	1	0	1	1
Diarrhoea (*Microsporidium* spp.)	0	0	0	0	1	1
Acquired syphilis	0	0	1	0	0	0
Nº. of patients with comorbidities (%)	0 (0)	0 (0)	6 (75) [C]	0 (0)	4 (50) [D]	4 (50)

[A] AD1, patients receiving ART with a high adherence rate; AD2, treatment-naïve patients recently diagnosed with HIV infection or patients with discontinued and subsequently reinitiated ART with a high adherence rate during follow-up; NA, patients receiving ART with deficient adherence. [B] Patients were evaluated at two different time points (t0–t1) with an average follow-up of 12 months. [C] Two AD2 patients presented with pneumonia-herpes zoster infection and pneumonia-oral candidiasis simultaneously. [D] One non-adherent patient presented with diarrhoea and oral candidiasis simultaneously.

Figure legends

Figure 1. Flow cytometry gating strategy and representative dot-plots of RTE naïve $CD4^+$ T cells in HIV-infected children and uninfected controls. Whole blood was stained with CD4, CD45RA and CD31 monoclonal antibodies and analysed by flow cytometry. A, An R1 gate was set on the lymphocytes based on the forward scatter (FSC) *versus* the side-scatter (SSC) dot plot. $CD4^+$ T cells were then selected (R2) in a side-scatter (SSC) *versus* CD4 dot-plot. B, The expression of $CD45RA^+CD31^+$ on $CD4^+$ T cells was then analysed by joining the R1 and R2 gates. Group A, subjects moderately or severely immunosuppressed; Group B, subjects with no evidence of immunosuppression, as defined in the Materials and Methods. The numbers indicate the percentages of $CD4^+CD45RA^+CD31^+$ T cells.

Figure 2. Frequency of RTE naïve T cells in HIV-infected paediatric patients under antiretroviral treatment. Whole blood was collected, stained with anti-CD4, anti-CD45RA and anti-CD31 monoclonal antibodies and analysed by flow cytometry. $CD4^+$ T cells were gated by side-scatter (SSC) *versus* CD4 dot-plot, and the frequency of $CD4^+CD45RA^+CD31^+$ T cells was measured according to $CD4^+$ T cell percentages. Boxes represent values between the 25th and 75th percentiles and medians; bars indicate 10th and 90th percentiles. A) Group A, subjects moderately or severely immunosuppressed; Group B, subjects with no evidence of immunosuppression, as defined in the Materials and Methods. ANOVA and the Student-Newman-Keuls test were applied for statistical analysis. B) The frequency of $CD45RA^+CD31^+CD4^+$ T cells was determined at two different time points during an average follow-up period of 12 months. HIV-infected children were grouped according to their levels of $CD4^+$ T cells and viral load at inclusion (i.e., time 0), as defined in the Materials and Methods. ** $P< 0.001$ vs. t1 AD2; * $P< 0.05$ *vs.* AD1; AD2 and NA groups, at time 0 and time 1 of follow-up. Paired and unpaired Student's test was used for statistical analysis.



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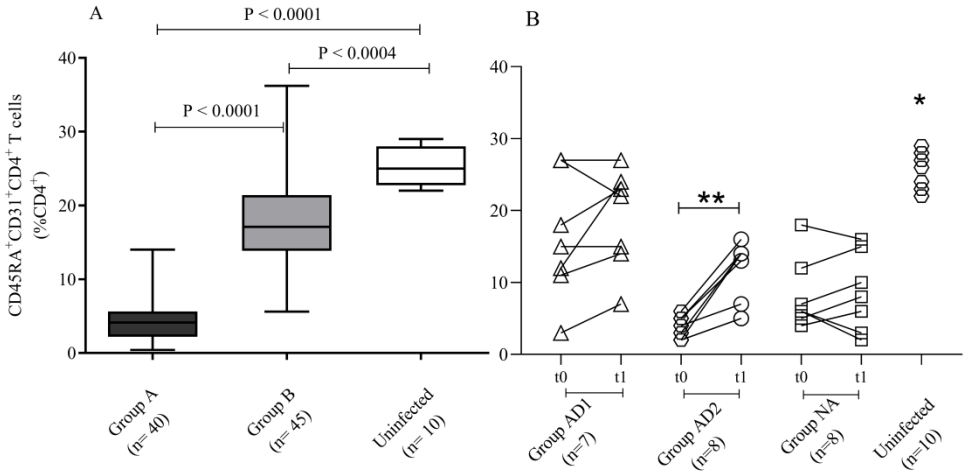

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