Naive T lymphocytes and recent thymic emigrants are associated with HIV-1 disease history in French adolescents and young adults infected in the perinatal period: the ANRS-EP38-IMMIP Study

Stéphane Blanche, Daniel Scott-Algara, Jérôme Le Chenadec, Céline Didier, Thomas Montange, Véronique Avettand-Fenoel, Christine Rouzioux, Adeline Mélard, Jean-Paul Viard, Catherine Dollfus, et al.

CID#72495-revised

Naive T lymphocytes and recent thymic emigrants are associated with HIV-1 disease history in French adolescents and young adults infected in the perinatal period: the ANRS-EP38-IMMIP Study

Stéphane Blanche[1,2], Daniel Scott-Algara[3], Jérôme Le Chenadec[4], Céline Didier[3], Thomas Montange[5,6], Véronique Avettand-Fenoel[1,7], Christine Rouzioux[1,7], Adeline Mélard[1,7], Jean-Paul Viard[1,8], Catherine Dollfus[9], Naima Bouallag[4], Josiane Warszawski[4,10], Florence Buseyne[5,6]

[1] EA3620, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France

[2] AP-HP, Unité Immunologie et Hématologie Pédiatrique, Hôpital Necker-Enfants Malades, Paris, France

[3] Institut Pasteur, Unité de Régulation des Infections Rétrovirales, Paris, France

[4] CESP INSERM U1018, Le Kremlin-Bicêtre, France

[5] Institut Pasteur, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Paris, France

[6] CNRS, UMR 3569, Paris, France

[7] AP-HP, Laboratoire de Virologie, Hôpital Necker-Enfants Malades, Paris, France

[8] AP-HP, Centre de Diagnostic et de Thérapeutique, Hôpital de l'Hôtel-Dieu, Paris, France

[9] AP-HP, Service d'Hématologie et d'Oncologie Pédiatrique, Hôpital Trousseau, Paris, France

[10] Université Paris-Sud, Le Kremlin-Bicêtre, France

Keywords: perinatal HIV infection, adolescents, naive T lymphocyte, thymus, CD4 T-cell count, cumulative viremia

Running title: Naive T cells in perinatal HIV infection

40-word summary: In youths infected with HIV during the perinatal period, $CD4_N$ and $CD4_{RTE}$ percentages were positively correlated with both CD4 T-cell count and past and current HIV replication, demonstrating the persistence of high-level thymic activity in long-term infection.

Corresponding author

Florence Buseyne, PhD, Institut Pasteur, Unité d'Epidemiologie et de Physiopathologie des Virus Oncogènes, Bat. Lwoff, 28 rue du Dr Roux, 75015 Paris, France. Phone: 33 1 45 68 88 99; Fax: 33 1 40 61 34 65; florence.buseyne@pasteur.fr

Alternative corresponding author

Stéphane Blanche, AP-HP, Unité Immunologie et Hématologie Pédiatrique, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, Paris, France. Phone: 33 1 44 49 48 24; Fax: 33 1 44 49 48 18; stephane.blanche@nck.aphp.fr

Tables: 2, Figures: 1

Word count: Summary (max 40):37, Abstract (max 250):244, Text (max 3000):3000

Background: Children born at the start of the HIV epidemic and infected during the perinatal period are now young adults living with the virus. Naive T-lymphocyte restoration is essential for the maintenance of a diverse TCR repertoire and for immunity to pathogens.

Methods: The ANRS-EP38-IMMIP study included 93 patients infected with HIV-1 during the perinatal period. Naive CD4 ($CD4_N$) and CD8 ($CD8_N$) T lymphocytes and CD4 recent thymic emigrants ($CD4_{RTE}$) were quantified in the peripheral blood by flow cytometry. Wilcoxon tests, Pearson's correlation coefficients and linear regressions were used to study their associations with HIV disease parameters.

Results: Median (IQR) $CD4_N$, $CD8_N$ and $CD4_{RTE}$ percentages were 56% (44-64), 31% (22-44) and 79% (74-83), respectively. The three T-lymphocyte subsets were positively correlated with CD4 T-cell count. Patients aviremic at the time of the study tended to have a lower $CD4_N$ percentage (55% vs. 58%, P=0.10), had a significantly higher $CD8_N$ percentage (39% vs. 22%, P<0.0001), and a significantly lower $CD4_{RTE}$ percentage (77% vs. 81%, P=0.003) than viremic patients. In aviremic patients, $CD4_N$ percentages were positively associated with cumulative viremia over the last 10 years (r=0.335, P=0.01) and were significantly higher in patients harboring X4R5 viruses than in those harboring R5 viruses (61 vs. 44%, P=0.001).

Conclusion: After at least 15 years of HIV infection, perinatally infected youths had preserved $CD4_N$ and $CD4_{RTE}$ levels. This persistence of high levels of thymic activity potentially compensating for the deleterious effects of current and past HIV replication is remarkable.

Introduction

The first generation of patients infected with HIV-1 during the perinatal period has now reached adolescence or adulthood [1-2]. Pediatric HIV infection differs from infection during adulthood in terms of clinical progression, pathophysiology, response to treatment, and the occurrence of viral replication during immune system development [2-3]. Adolescence may be associated with poorer compliance with treatment in some patients, and physiological changes during puberty may affect the immune system [4]. The current immune and virological status of youths perinatally infected with HIV and their covariates have been the subject of limited investigations. The restoration and maintenance of the naive T-lymphocyte compartment is of importance for the future of these individuals, as it is directly associated with the diversity of the T-cell receptor (TCR) repertoire and critical for immunity to foreign antigens [5]. Previous pediatric studies have shown that CD4 T-lymphocyte recovery is driven principally by the *de novo* production of naive T lymphocytes in the thymus, and that it declines with age and with HIV infection [6-7].

Our aim, within the ANRS-EP38-IMMIP study, was to provide more detailed knowledge of the pathophysiological characteristics of youths, 15 years or more after perinatal infection. We previously reported that blood cell-associated HIV-DNA levels in these youths, aged 15-24 years, were similar to those in infected adults. However, contrary to findings for adults, we found no negative correlation between HIV-DNA level and CD4 T-cell counts in patients with suppressed viral replication [8]. This pattern of immunovirological equilibrium was observed in children and may result from sustained naive CD4 T-lymphocyte production by the thymus. We now report on the levels of naive CD4 ($CD4_N$) and CD8 ($CD8_N$) T lymphocytes, and CD4 recent thymic emigrants ($CD4_{RTE}$) in the HIV-infected youths included in the ANRS-EP38-IMMIP study, and on the HIV disease factors associated with these three T-lymphocyte subsets.

Patients and methods

Patients

The characteristics of the 93 HIV-infected youths included in the ANRS-EP38-IMMIP are presented in Table 1 for the aviremic and viremic groups. The study was approved by the local ethics committees. All patients, and their legal guardians for those under 18 years of age, received written information and signed an informed consent form. The inclusion criteria were (1) HIV-1 infection following vertical transmission, (2) aged over 15 years, (3) no change in treatment status (treated or untreated) during the previous six months, and (4) introduction of care for HIV infection before 1996, to ensure that all patients had similar access to HAART. A single 25 ml blood sample was taken for biological evaluations.

T-lymphocyte phenotyping

CD4 and CD8 T-lymphocyte phenotypes were determined on fresh whole blood for 82 patients, using combinations of the following antibodies: CD28-FITC, CD4-RD1, CD45RA-ECD, CD45RO-ECD, CD62L-PC5, CD27-PC5, CD4-PC7, and CD8-PC7 (Beckman Coulter, Villepinte, France). Data were collected on an FC500 cytometer and analyzed with CXP software (Beckman Coulter). $CD4_N$ levels were defined as the percentage of CD4 T lymphocytes that were $CD45RA^+CD62L^+$, $CD8_N$ levels as the percentage of CD8 T lymphocytes that were $CD45RO^-CD28^+CD27^+$. For 57 patients, frozen PBMCs were available for the quantification of $CD4_{RTE}$, with Live-Dead-Aqua (Life Technologies, Saint-Aubin, France) labeling of dead cells, and the following antibodies: CD3-ECD and CD31-FITC (Beckman Coulter), CD4-APC-efluor780 (e-bioscience, Paris, France), CD45RA-V450 and CD197-PC7 (BD Biosciences, Rungis, France). Data were collected on an LSR II cytometer (BD Biosciences) and analyzed with Flow-Jo software (Treestar, Ashland, USA). $CD4_{RTE}$ levels were defined as the percentage of naive $CD45RA^+CCR7^+CD4^+$ T lymphocytes positive for CD31.

Virological assays

HIV-1 reverse transcriptase (RT) and V3 Env sequences were obtained from blood cell HIV-DNA as previously described (http://www.hivfrenchresistance.org/). HIV-1 B and non-B subtypes were defined by phylogenetic analysis (http://www.hiv-web.lanl.gov). HIV-1 coreceptor usage was determined with the SVMGeno2pheno algorithm, with a 5.75% false positive rate [9]. HIV-RNA was quantified at the clinical sites, whereas HIV-DNA was quantified at a central laboratory, by real-time PCR with the Generic HIV-DNA Cell kit (Biocentric, Bandol, France) [8]. Serological tests for CMV were carried out on frozen plasma, with the CMV IgG Liaison II kit (Diasorin, Antony, France).

Statistical analysis

A full description of study variables has been provided elsewhere [8]. We studied univariate associations between T-lymphocyte subsets and variables defining current and past HIV disease, using the Wilcoxon test for categorical variables, and Pearson's correlation coefficients for continuous variables. Analyses were stratified on the basis of plasma HIV-RNA detection, using a cutoff value of 80 HIV-RNA copies/ml to define aviremic and viremic patients. Multivariate analyses were performed by linear regression. Separate models were built for aviremic and viremic patients, due to interactions between plasma HIV-RNA detection and variables describing HIV disease history (data not shown). For each dependent variable, one full model was adjusted for non collinear variables associated with the dependent variable in univariate analysis at P <0.20. Age, as exposure factor of interest was systematically included in all models, as was current HIV-RNA level for viremic patients, whatever the *P*-value. Economical models including only variables with P values <0.05 gave similar results, supporting the stability of analyses (data not shown). Variables non included in model because of collinearity are indicated in tables footnotes. Analyses were conducted with SAS statistical software (version 9.2). P<0.05 defined statistical significance.

Results

$CD4_N$, $CD8_N$, and $CD4_{RTE}$ T-lymphocyte levels differ in their association with current viral replication

Median percentages were 56% for $CD4_N$ (interquartile range, [IQR], 44-64%), and 31% for $CD8_N$ (IQR, 22-44%). Patients who were aviremic at the time of the study tended to have a lower $CD4_N$ percentage (55% vs. 58%, *P*=0.10), and had a significantly higher $CD8_N$ percentage (39% vs. 22%, *P*<0.0001), than viremic patients (Figure 1A, 1B). Naive T lymphocytes may be generated by the thymus or by peripheral proliferation. We investigated their origin by quantifying a correlate of thymic activity: the percentage of $CD31^+$ $CD4_{RTE}$ among naive CD4 T lymphocytes [10]. The median $CD4_{RTE}$ percentage was 79% (IQR, 74-83%). Aviremic patients had a significantly lower $CD4_{RTE}$ percentage than viremic patients (77% vs. 81%, *P*=0.003, Figure 1C). Thus, despite their shared developmental pathway, $CD4_N$, $CD8_N$ and $CD4_{RTE}$ naive T lymphocytes are differentially associated with current HIV replication.

Associations between $CD4_N$, $CD8_N$, and $CD4_{RTE}$ T-lymphocyte levels and HIV disease history in aviremic patients

$CD4_N$, $CD8_N$ and $CD4_{RTE}$ percentages were positively correlated with CD4 T-cell count in aviremic patients ($CD4_N$ r=0.431, *P*=0.001; $CD8_N$: r=0.463, *P*=0.0007; $CD4_{RTE}$: r=0.520, *P*=0.0007, Figure 1G-I), and with the CD4 T-cell percentage and CD4/CD8 ratio (Table 2). $CD4_N$ and $CD8_N$ percentages were positively correlated with each other (r=0.607, *P*<0.0001, Figure 1D), and were not correlated with $CD4_{RTE}$ percentage ($CD4_N$: r=0.278, *P*=0.12; $CD8_N$: r=0.226, *P*=0.18, Figure 1E-F). None of the three T-lymphocyte subsets was associated with ethnicity, HIV subtype, CDC stage, age at the time of the study, age at the time of nadir CD4 T-cell percentage, or age at the initiation of first HAART (Table 2).

$CD4_N$ percentage was positively correlated with cumulative viremia over the last 10 years (r=0.335, *P*=0.01). It also tended to be positively correlated with HIV-DNA level in PBMCs, and negatively correlated with the duration of the last period during which plasma HIV-RNA level was below 500 copies/ml (Table 2). Patients harboring X4R5 viruses had a significantly higher $CD4_N$ percentage than patients harboring R5 viruses (medians: 44% vs. 61%, *P*=0.001). $CD4_N$ percentage was not associated with sex, CMV seropositivity, nadir CD4 T-cell percentage, or the time for which CD4 T-cell percentage was below 15% during the lifetime of the patient (Table 2).

$CD8_N$ percentage was significantly higher in female than in male patients (medians: 45% vs. 33%, respectively, *P*=0.01), and was significantly higher in CMV-seronegative patients than in CMV-seropositive patients (medians: 45% vs. 31%, respectively, *P*=0.0001). There was a trend towards a higher $CD8_N$ percentage in patients with X4R5 viruses than in those with R5 viruses (medians: 45% vs. 35%, respectively *P*=0.10), and towards a negative correlation with the cumulative duration of HAART over the last 10 years (r=-0.263, *P*=0.06). $CD8_N$ percentage was not correlated with past HIV-RNA levels or past immunosuppression (Table 2).

$CD4_{RTE}$ percentage was higher in female than in male patients (79% vs. 73%, respectively, *P*=0.002), and CMV-seropositive patients tended to have a lower $CD4_{RTE}$ percentage than CMV-seronegative patients (74% vs. 79%, *P*=0.11, Table 2). $CD4_{RTE}$ percentage was positively correlated with nadir CD4 T-cell percentage and negatively correlated with the time for which CD4 T-cell percentage was below 15% during the patient's lifetime (r=0.456, *P*=0.004, and r=-0.446, *P*=0.004, respectively). $CD4_{RTE}$ percentage was not associated with past HIV-RNA levels or HIV coreceptor usage (Table 2).

In multivariate analysis, $CD4_N$ percentage was independently associated with CD4 T-cell count, X4 coreceptor usage and tended to be associated with cumulative viremia over the

last 10 years, after adjustment for age and CDC stage (Table 2). $CD8_N$ percentage was independently associated with coreceptor usage and sex, and tended to be associated with CD4 T-cell count after adjustment for age, CMV serostatus and cumulative duration of HAART over the last 10 years. $CD4_{RTE}$ percentage was independently associated with CD4 T-cell count, sex, and time for which CD4 T-cell percentage was below 15% during the patient's lifetime, after adjustment for age and CMV serostatus.

Associations between $CD4_N$, $CD8_N$, and $CD4_{RTE}$ T-lymphocyte levels and HIV disease history in viremic patients

In viremic patients, $CD4_N$ and $CD8_N$ percentages were directly correlated with CD4 T-cell count (r=0.393, *P*=0.04 and r=0.749, *P*<0.0001, respectively, Figure 1G-H), and with each other (r=0.435, *P*=0.02, Figure 1D). $CD4_N$ and $CD8_N$ percentages were not correlated with plasma HIV-RNA level (r=-0.021, *P*=0.91 and r=-0.285, *P*=0.13, respectively). $CD4_N$ percentage was not associated with demographic factors, viral characteristics or past HIV disease parameters (Table 3). $CD8_N$ percentage was associated with CMV serostatus (medians: 27% vs. 20% in seronegative and seropositive patients, *P*=0.03), and negatively correlated with cumulative viremia over the last 10 years (r=-0.382, *P*=0.04), and HIV-DNA level (r=-0.397, *P*=0.04). $CD4_{RTE}$ percentage was positively correlated with $CD4_N$ percentage (r=0.762, *P*=0.0006, Figure 1E), but not with $CD8_N$ percentage (r=0.132, *P*=0.64, Figure 1F). It was not correlated with CD4 T-cell count (r=-0.032, *P*=0.90, Figure 1H), plasma HIV-RNA level (r=-0.125, *P*=0.62) or the other study variables.

In multivariate analysis, both $CD4_N$ and the $CD8_N$ percentages were independently associated only with CD4 T-cell count (Table 3). In an alternative model, the $CD8_N$ percentage was associated only with CD4 T-cell count after adjustment for age at nadir CD4 T-cell %, cumulative viremia and CMV seropositivity (data not shown).

Discussion

We found that, 15 or more years after perinatal infection, naive CD4 T-lymphocyte levels and thymic activity were preserved and positively correlated with current CD4 T-cell count. High levels of viral replication and/or viral cytopathogenicity over the patient's lifetime were associated with higher levels of naive CD4 T lymphocytes at the time of the study.

The $CD4_N$ percentages of our study population were in the range reported for uninfected individuals, corresponding to half to two thirds of total CD4 T lymphocytes [11-12]. $CD4_{RTE}$ values were also similar to those reported for healthy young adults [13]. Our results extend those of a previous study on 20 perinatally infected youths on suppressive HAART, which reported normal levels of CD4 T-lymphocyte subsets and thymic activity [12]. $CD8_N$ T-lymphocyte percentages were slightly lower than those reported for healthy young adults in our aviremic patients and were lower still in viremic patients [14]. The different patterns of CD4 and CD8 T-lymphocyte restoration reflect the more robust expansion of memory CD8 than of memory CD4 T lymphocytes in response to HIV replication, chemotherapy-induced lymphodepletion and aging [6, 15-16].

$CD4_{RTE}$ percentage was directly correlated with total CD4 T-lymphocyte count, as were the $CD4_N$ and $CD8_N$ percentages. In patients infected during adulthood, immune restoration involves both thymus-driven T-lymphocyte production and homeostatic proliferative expansion, and the failure of CD4 T-cell restoration despite prolonged viral suppression is associated with defective thymus function [17-18]. Two of the 93 patients included in the ANRS-EP38-IMMIP study had current CD4 T-cell counts below 350 cells/µl, despite being aviremic. One patient had recently experienced viral blips, whereas the other had remained continuously aviremic for more than six years and was, thus, the only patient that could have been classified as a "low CD4 responder" [18]. Overall, our results highlight

the active role of the thymus in both qualitative and quantitative immune restoration in the patients studied.

One key finding of this study was that, in patients in which HIV replication was suppressed at the time of the study, higher $CD4_N$ levels were associated with higher levels of viral replication in the previous 10 years. Furthermore, there was a trend towards higher $CD4_N$ percentages in aviremic than in viremic patients. The results are expressed as percentages, and this association may therefore reflect either an increase in the production of naive T lymphocytes or higher levels of memory CD4 T-lymphocyte destruction in the presence of viral replication. In this second case, higher levels of viral replication would be associated with a higher $CD4_N$ percentage and a lower CD4 T-cell count, and we would expect a negative correlation between $CD4_N$ and CD4 T-cell count to be observed. By contrast, $CD4_N$ percentage and CD4 T-cell count were positively correlated. Thus, the positive correlation between $CD4_N$ percentage and cumulative viremia probably reflects higher levels of thymic output during previous periods of HIV replication. Indeed, high levels of thymic activity have been associated with high HIV-RNA and HIV-DNA levels in pediatric patients on HAART [19-20]. A positive correlation between blood HIV-DNA and TREC levels has been reported in children with treatment-induced viral suppression [21]. These findings have been interpreted as indicating that *de novo* T-lymphocyte production by the thymus is a homeostatic response to naive CD4 T-lymphocyte destruction and/or recruitment to the memory compartment driven by HIV replication. Our results are entirely consistent with this explanation and extend observations on children by showing the cumulative effect of viral replication on $CD4_N$ T-lymphocyte levels 15 or more years after infection.

Among patients with suppressed viral replication, $CD4_N$ and $CD8_N$ percentages were higher in patients harboring X4R5 viruses than in those with R5 viruses. This association is

counterintuitive, as X4R5 viruses infect thymocytes and $CD4_N$ T lymphocytes more efficiently than R5 viruses, because of the more widespread expression of CXCR4 on these cells [17]. Given that these differences were observed only in aviremic patients, the detection of X4R5 viruses by cell-associated HIV-DNA sequencing of archived virus may reflect past events, such as deeper immunosuppression associated with higher levels of viral replication, and/or infection with more cytopathogenic viruses [22]. However, potent peripheral immune restoration has been observed in children with severe immunosuppression at treatment initiation [23-25], and the association between $CD4_N$ percentage and viral tropism is consistent with that between $CD4_N$ percentage and cumulative viremia.

$CD4_{RTE}$ percentage was higher in viremic than in aviremic patients. This association may reflect the confounding effect of disease severity. However, viremic and aviremic youths did not differ in terms of previous occurrence of CDC stage C events, nadir CD4 T-cell percentage, or age at first HAART initiation (data not shown). In patients with active viral replication, $CD4_{RTE}$ levels may increase because these cells are more resistant to HIV infection than their CD31-negative counterparts [26-27]. Finally, this association may simply reflect an increase in thymic activity to maintain T-cell homeostasis in the presence of the virus, as discussed previously. In untreated adults, several studies have shown that active thymopoiesis occurs during the early asymptomatic phase of HIV infection, at levels above those in uninfected controls [28-30]. In the early HAART period, $CD4_N$ T-lymphocyte reconstitution was reported in children with a partially reduced viral load [19, 31-32]. Overall, our data suggest that thymic activity compensates for the destructive effect of viral replication, even after childhood. This result and the lack of correlation between age and $CD4_{RTE}$ percentage contrast with the decline of blood thymic excision circles reported in uninfected teenagers and young adults [33]. Importantly, in patients with suppressed viral replication at the time of the study, $CD4_{RTE}$ levels were negatively correlated with the

duration of severe immunosuppression (i.e., a CD4 T-cell percentage below 15%), suggesting that the thymus remains active, but has been damaged by past viral replication.

$CD8_N$ and $CD4_{RTE}$ levels were higher in female than in male patients, consistent with the higher levels of thymic activity reported for women than for men in studies of healthy donors [34-35]. However, it remains unclear why $CD4_N$ levels were not affected by sex. CMV infection induces a large expansion of memory T lymphocytes and a decrease in the size of the naive T-cell compartment [36]. $CD8_N$ percentages were, indeed, lower in CMV-seropositive than in CMV-seronegative patients in univariate analysis. However, this association was not statistically significant in multivariate analysis.

One limitation of our study is that treatment status was heterogeneous in the viremic patients (HAART naive, HAART interruption, HAART without viral suppression). The small numbers of patients in each category precluded independent analysis. Therefore, comparisons of patients with and without viral suppression should be interpreted with caution. The associations between higher levels of viral replication and higher levels of naive T lymphocytes were convincingly confirmed by analyses of viral history in patients who were aviremic at the time of the study. CD31 expression on naive CD4 T cells is a convenient, but imperfect marker of thymic activity. $CD4_{RTE}$ cells may undergo proliferation in the periphery before losing CD31 expression, a process subject to interindividual and age-dependent variation [37]. However, limitations on the volumes of blood that can be drawn from adolescents have limited our ability to perform additional tests, such as TREC or telomere length assessment. For the same reasons, $CD4_{RTE}$ data from frozen PBMC samples were available for only 60% of the patients, reducing the statistical power of the analyses.

Our data for this first generation of patients infected with HIV in the perinatal period without early access to HAART are encouraging for the future clinical management of these patients. They are also consistent with the good health status reported for perinatally infected

youths living in France [1]. The paradoxically high level of thymic activity in subjects with sustained viral replication provides an argument for longitudinal follow-up of the naive T-cell compartment in these perinatally infected youths.

Funding

This work was supported by the « Agence Nationale de la Recherche sur le SIDA et les Hépatites virales » (ANRS) and the « Fondation AREVA ».

Acknowledgments

We thank all the patients who agreed to participate in this study. We would also like to thank the nurses and staff members from the various clinical sites. We thank Sandrine Leveillé (Hôpital Robert Debré), Geneviève Vaudre (Hôpital Trousseau), Sylvie Tassi (Hôpital Jean Verdier), Nora Boudjoudi (Hôpital Port Royal), Marie-Christine Mourey (Hôpital Necker), Thierry Wack (CESP INSERM U1018), and Yassine Benmebarek (former member of CESP INSERM U1018). We are indebted to Yves Rivière for his role in this collaborative work. This text has been verified by a native English speaker. The authors have no conflict of interest to declare.

APPENDIX

This study was approved by the "Comité de protection des personnes Ile-de-France II" (registration number 06-09-08), authorized by the "Direction Générale de la Santé" (authorization number 2006-AOO142-49), and registered as an observational study at www.clinicaltrials.gov under identifier NCT01055873.

The institutions and investigators of the ANRS-EP38-IMMIP Study were: Pédiatrie-néonatologie, Hôpital Louis Mourier, Colombes (Corinne Floch-Tudal); Gynécologie-Obstétrique, Groupe Hospitalier Cochin Tarnier-Port-Royal, Paris (Ghislaine Firtion); Pédiatrie-Centre Hospitalier Intercommunal, Créteil (Sophie Lemerle); Pédiatrie-Centre Hospitalier Intercommunal, Villeneuve Saint-Georges (Anne Chace); Immuno-Hématologie Pédiatrique, Groupe Hospitalier Necker-Enfants Malades, Paris (Stéphane Blanche, Florence Veber); Pédiatrie, Centre Hospitalier Sud-Francilien, Evry (Adrien May); Maladies Infectieuses, Hôpital Jean Verdier, Bondy (Vincent Jeantils); Onco-Hématologie Pédiatrique

Hôpital Trousseau, Paris (Catherine Dollfus); Pédiatrie-Hôpital Robert Debré, Paris (Martine Levine, Albert Faye); Centre de Diagnostic et de Thérapeutique, Hôpital de l'Hôtel-Dieu, Paris (Jean-Paul Viard).

References

1. Dollfus, C., J. Le Chenadec, A. Faye, et al., Long-term outcomes in adolescents perinatally infected with HIV-1 and followed up since birth in the French Perinatal Cohort (EPF/ANRS CO10). Clin Inf Dis, 2010. 51: 214-24.

2. Hazra, R., G.K. Siberry, and L.M. Mofenson, Growing up with HIV: children, adolescents, and young adults with perinatally acquired HIV infection. Ann Rev Med, 2010. 61: 169-85.

3. Tobin, N.H. and G.M. Aldrovandi, Immunology of pediatric HIV infection. Immunol Rev, 2013. 254: 143-69.

4. Fish, E.N., The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol, 2008. 8: 737-44.

5. Wils, E.J., B. van der Holt, A.E.C. Broers, et al., Insufficient recovery of thymopoiesis predicts for opportunistic infections in allogeneic hematopoietic stem cell transplant recipients. Haematol-Hematol J, 2011. 96: 1846-54.

6. Douek, D.C., L.J. Picker, and R.A. Koup, T cell dynamics in HIV-1 infection. Ann Rev Immunol, 2003. 21: 265-304.

7. Hudson, L.L., M.L. Markertac, B.H. Devlin, B.F. Haynes, and G.D. Sempowski, Human T cell reconstitution in DiGeorge syndrome and HIV-1 infection. Sem Immunol, 2007. 19: 297-309.

8. Avettand-Fenoel, V., S. Blanche, J. Le Chenadec, et al., Relationships between HIV disease history and blood HIV-1 DNA load in perinatally infected adolescents and young adults: the ANRS-EP38-IMMIP Study. J Infect Dis, 2012. 205: 1520-8.

9. Frange, P., L. Meyer, J. Ghosn, et al., Prevalence of CXCR4-tropic viruses in clustered transmission chains at the time of primary HIV-1 infection. Clin Mic Infect, 2013. 19: E252-E5.

10. Kohler, S. and A. Thiel, Life after the thymus: CD31+ and CD31- human naive CD4+ T-cell subsets. Blood, 2009. 113: 769-74.

11. Shearer, W.T., H.M. Rosenblatt, R.S. Gelman, et al., Lymphocyte subsets in healthy children from birth through 18 years of age: The pediatric AIDS clinical trials group P1009 study. J Aller Clin Immunol, 2003. 112: 973-80.

12. Lee, J.C., M.I. Boechat, M. Belzer, et al., Thymic volume, T-cell populations, and parameters of thymopoiesis in adolescent and adult survivors of HIV infection acquired in infancy. AIDS, 2006. 20: 667-74.

13. Kimmig, S., G.K. Przybylski, C.A. Schmidt, et al., Two subsets of naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. J Exp Med, 2002. 195: 789-94.

14. Shearer, W.T., H.M. Rosenblatt, S. Spector, et al., Age-related expression of naive (CD45RA/62L) and activation (HLA DR/CD38) surface markers on CD4+ and CD8+ T-cell in normal children (birth to 18 years). J Aller Clin Immunol, 2002. 109: S199-S.

15. Mackall, C.L., T.A. Fleisher, M.R. Brown, et al., Distinctions between CD8+ and CD4+ T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. Blood, 1997. 89: 3700-7.

16. Di Mascio, M., I. Sereti, L.T. Matthews, et al., Naive T-cell dynamics in human immunodeficiency virus type 1 infection: Effects of highly active antiretroviral therapy provide insights into the mechanisms of naive T-cell depletion. J Virol, 2006. 80: 2665-74.

17. Fang, R.H.T., A.D. Colantonio, and C.H. Uittenbogaart, The role of the thymus in HIV infection: a 10 year perspective. AIDS, 2008. 22: 171-84.

18. Gazzola, L., C. Tincati, G.M. Bellistri, A.D. Monforte, and G. Marchetti, The absence of CD4+ T-cell count recovery despite receipt of virologically suppressive highly

active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. Clin Inf Dis, 2009. 48: 328-37.

19. De Rossi, A., A.S. Walker, N. Klein, et al., Increased thymic output after initiation of antiretroviral therapy in human immunodeficiency virus type 1-infected children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial. J Infect Dis, 2002. 186: 312-20.

20. De Rossi, A., A.S. Walker, D. De Forni, N. Klein, and D.M. Gibb, Relationship between changes in thymic emigrants and cell-associated HIV-1 DNA in HIV-1-infected children initiating antiretroviral therapy. Antiv Ther, 2005. 10: 63-71.

21. Saitoh, A., K.K. Singh, S. Sandall, et al., Association of CD4+ T-lymphocyte counts and new thymic emigrants in HIV-infected children during successful highly active antiretroviral therapy. J Aller Clin Immunol, 2006. 117: 909-15.

22. Koot, M., I.P.M. Keet, A.H.V. Vos, et al., Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med, 1993. 118: 681-8.

23. Vigano, A., S. Vella, M. Saresella, et al., Early immune reconstitution after potent antiretroviral therapy in HIV-infected children correlates with the increase in thymus volume. AIDS, 2000. 14: 251-61.

24. Hainaut, M., M. Ducarme, L. Schandene, et al., Age-related immune reconstitution during highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children. Ped Inf Dis J, 2003. 22: 62-9.

25. Essajee, S.M., M. Kim, C. Gonzalez, et al., Immunologic and virologic responses to HAART in severely immunocompromised HIV-1-infected children. AIDS, 1999. 13: 2523-32.

26. Brenchley, J.M., B.J. Hill, D.R. Ambrozak, et al., T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: Implications for HIV pathogenesis. J Virol, 2004. 78: 1160-8.

27. Delobel, P., M.T. Nugeyre, M. Cazabat, et al., Naive T-cell depletion related to infection by X4 human immunodeficiency virus type 1 in poor immunological responders to highly active antiretroviral therapy. J Virol, 2006. 80: 10229-36.

28. Nobile, M., R. Correa, J.A.M. Borghans, et al., De novo T-cell generation in patients at different ages and stages of HIV-1 disease. Blood, 2004. 104: 470-7.

29. McCune, J.M., R. Loftus, D.K. Schmidt, et al., High prevalence of thymic tissue in adults with human immunodeficiency virus-1 Infection. J Clin Invest, 1998. 101: 2301-8.

30. Dion, M.L., J.F. Poulin, R. Bordi, et al., HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. Immunity, 2004. 21: 757-68.

31. Sleasman, J.W., R.P. Nelson, M.M. Goodenow, et al., Immunoreconstitution after ritonavir therapy in children with human immunodeficiency virus infection involves multiple lymphocyte lineages. J Pediatr, 1999. 134: 597-606.

32. Chavan, S., B. Bennuri, M. Kharbanda, et al., Evaluation of T cell receptor gene rearrangement excision circles after antiretroviral therapy in children infected with human immunodeficiency virus. J Infect Dis, 2001. 183: 1445-54.

33. Zhang, L., S.R. Lewin, M. Markowitz, et al., Measuring recent thymic emigrants in blood of normal and HIV-1–infected individuals before and after effective therapy. J Exp Med, 1999. 190: 725-32.

34. Mitchell, W.A., P.O. Lang, and R. Aspinall, Tracing thymic output in older individuals. Clin Exp Immunol, 2010. 161: 497-503.

35. Pido-Lopez, J., N. Imami, and R. Aspinall, Both age and gender affect thymic output: more recent thymic migrants in females than males as they age. Clin Exp Immunol, 2001. 125: 409-13.

36. Moss, P., The emerging role of cytomegalovirus in driving immune senescence: a novel therapeutic opportunity for improving health in the elderly. Curr Opin Immunol, 2010. 22: 529-34.

37. Kilpatrick, R.D., T. Rickabaugh, L.E. Hultin, et al., Homeostasis of the naive CD4+ T cell compartment, during aging. J Immunol, 2008. 180: 1499-507.

38. Centers for Disease Control and Prevention, 1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. MMWR, 1994. 43: 1-10.

39. Centers for Disease Control and Prevention, Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR, 1992. 41: 1-19.

40. Zoufaly, A., H.J. Stellbrink, M. an der Heiden, et al., Cumulative HIV Viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. J Infect Dis, 2009. 200: 79-87.

Table 1: Characteristics of patients as a function of plasma HIV RNA detection

		Aviremic patients	**Viremic patients**
		***n*=59**	***n*=34**
		% (n)	% (n)
Sociodemographic, clinical and virological variables			
Sex	Male	55.9 (33)	58.8 (20)
	Female	44.1 (26)	41.2 (14)
Ethnicity [a]	Black	33.9 (20)	48.5 (16)
	Other	66.1 (39)	51.5 (17)
CDC stage [b]	Non C	71.2 (42)	82.4 (28)
	C	28.8 (17)	17.6 (6)
HIV-1 subtype	Non B	21.1 (12)	44.1 (15)
	B	78.9 (45)	55.9 (19)
HIV-1 coreceptor usage	R5	52.8 (28)	66.7 (22)
	X4R5	47.2 (25)	33.3 (11)
anti-CMV IgG	Negative	50.8 (30)	29.4 (10)
	Positive	49.2 (29)	70.6 (24)
		Median (IQR)	Median (IQR)
Current status			
Age (years)		18 (15-19)	16 (15-19)
HIV-RNA (log_{10} copies/ml)		NA	3.7 (3.0-4.4)
HIV-DNA (log_{10} copies/10^6 PBMCs)		2.8 (2.4-3.0)	3.1 (2.8-3.5)
CD4 T-cell count/µl		642 (522-944)	423 (270-534)
CD4 T-cell percentage		31 (27-36)	21 (14-27)
CD4/CD8 ratio		0.85 (0.68-1.17)	0.38 (0.27-0.55)
HIV disease history			
Age at first HAART [c] (years)		7 (5-10)	8 (6-10)
Cumulative duration of HAART over the last 10 years (months)		108 (88-118)	83 (52-112)
Duration of last period of HIV RNA < 500 copies/ml (months)		47 (17-75)	NA

Cumulative viremia over the last 10 years [d]	4489 (2161-6221)	10415 (6139-12009)
Nadir CD4 T-cell % [e]	8 (5-10)	9 (4-13)
Age at nadir CD4 T-cell % (years)	8 (2-14)	9 (3-11)
Duration of CD4 T-cell % < 15 during lifetime (months)	23 (5-50)	28 (5-56)

Abbreviations: IQR, interquartile range; CDC, Centers for Disease Control and Prevention; R5, virions using CCR5 as a coreceptor; X4R5, virions using CXCR4 as a coreceptor and dual-tropic viruses; CMV, cytomegalovirus; NA, not applicable; HAART, highly active antiretroviral therapy

[a] patients whose mothers originated from Sub-Saharan Africa and the Caribbean were grouped together and compared with those born to mothers with other geographic origins (mainland France, North Africa and Asia)

[b] according to the Centers for Disease Control and Prevention (CDC) classification [38-39]

[c] HAART was defined as any combination of at least three drugs or any combination including at least one protease inhibitor or one non nucleoside reverse transcriptase inhibitor.

[d] Cumulative viremia was defined as the area under the curve of HIV-RNA load over time and was estimated as previously described [40]. Results are expressed as days x $\log_{10}$ HIV-RNA copies/ml of plasma.

[e] nadir was defined as the lowest value reached during follow-up

Table 2: Univariate and multivariate analysis of factors associated with $CD4_N$, $CD8_N$ and $CD4_{RTE}$ percentages in aviremic patients

Aviremic patients **Dependent variable: $CD4_N$**			**Unadjusted analysis[a]**			**Adjusted analysis[b]**		
		Median (IQR) Pearson's r	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
Sociodemographic, clinical and virological variables								
Sex	Male	53 (42-65)	Reference					
	Female	55 (43-63)	0.28	[-6.83;7.39]	0.94			
Ethnicity	Black	50 (39-67)	Reference					
	Other	55 (44-64)	2.07	[-5.21;9.35]	0.57			
CDC stage	Non C	53 (43-62)	Reference			Reference		
	C	61 (49-68)	5.59	[-2.48;13.66]	0.17	-0,48	[-9.24;8.27]	0.91
HIV-1 subtype	Non B	42 (37-59)	Reference					
	B	56 (44-64)	6.26	[-2.13 ;14.65]	0.14			
HIV-1 coreceptor usage	R5	44 (37-58)	Reference			Reference		
	X4R5	61 (52-68)	12.01	[5.26;18.76]	0.0008	10.38	[2.86;17.89]	0.008
anti-CMV IgG	Negative	56 (44-64)	Reference					
	Positive	51 (37-64)	-4.37	[-11.35;2.59]	0.21			

Current status							
Age [c]	-0.092	-0.52	[-2.09;1.05]	0.51	-0.36	[-1.80;1.08]	0.62
HIV-DNA [d]	0.239	6.26	[-0.91;13.43]	0.09			
CD4 T-cell count/µl [e]	0.431	2.21	[0.91;3.51]	0.001	1.64	[0.33;2.94]	0.02
CD4 T-cell percentage	0.292	0.54	[0.04;1.03]	0.03			
CD4/CD8 ratio	0.344	13.01	[3.77;22.23]	0.007			
HIV disease history							
Age at first HAART [c]	-0.069	-0.23	[-1.16;0.70]	0.62			
Cumulative duration of HAART over the last 10 years [f]	-0.114	-0.56	[-1.92;0.81]	0.42			
Duration of last period of HIV RNA < 500 copies/ml [f]	-0.221	-0.80	[-1.80;0.19]	0.11			
Cumulative viremia over the last 10 years [g]	0.335	13.26	[-2.70;23.80]	0.01	9.73	[-0.28;19.73]	0.06
Nadir CD4 T-cell %	0.014	0.02	[-0.49;0.54]	0.92			
Age at nadir CD4 T-cell % [c]	-0.013	-0.05	[-1.00;0.91]	0.92			
Duration of CD4 T-cell % < 15 during lifetime [f]	-0.164	-0.61	[-1.64;4.21]	0.24			

Aviremic patients
Dependent variable: $CD8_N$

			Unadjusted analysis[a]			Adjusted analysis[h]		
		Median (IQR)	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
		Pearson's r						
Sociodemographic, clinical and virological variables								
Sex	Male	33 (22-42)	Reference			Reference		
	Female	45 (33-53)	10.72	[2.90;18.53]	0.008	10.92	[3.08;18.77]	0.008
Ethnicity	Black	36 (27-52)	Reference					
	Other	41 (30-50)	1.35	[-7.49;10.19]	0.76			
CDC stage	Non C	36 (30-48)	Reference					
	C	41 (28-56)	5.18	[-3.84;14.20]	0.25			
HIV-1 subtype	Non B	30 (23-41)	Reference					
	B	42 (31-50)	7.65	[-2.70;18.00]	0.14			
HIV-1 coreceptor usage	R5	35 (28-47)	Reference			Reference		
	X4R5	45 (32-57)	7.77	[-0.98;16.53]	0.08	10.25	[2.58;17.92]	0.01
anti-CMV IgG	Negative	45 (34-54)	Reference			Reference		
	Positive	31 (23-43)	-11.08	[-18.83;-3.34]	0.006	-4.75	[-12.49;2.98]	0.22

Current status							
Age [c]	-0,045	-0,31	[-2.30;1.69]	0.76	-0.56	[-2.24;1.12]	0.51
HIV-DNA [d]	0.222	6.29	[-1.73;14.30]	0.12			
CD4 T-cell count/µl [e]	0.463	2.70	[1.20;4.20]	0.0007	1.47	[-0.05; 2.99]	0.06
CD4 T-cell percentage	0.358	0.74	[0.18;1.30]	0.01			
CD4/CD8 ratio	0.409	15.93	[5.50;26.36]	0.004			
HIV disease history							
Age at first HAART [c]	0.111	0.43	[-0.68;1.54]	0.44			
Cumulative duration of HAART over the last 10 years [f]	-0.263	-0.15	[-0.30;0.10]	0.07	-0.96	[-2.39;0.47]	0.18
Duration of last period of HIV RNA < 500 copies/ml [f]	-0.020	-0.08	[-1.28;1.10]	0.89			
Cumulative viremia over the last 10 years [g]	0.185	8.57	[-0.47;21.90]	0.20			
Nadir CD4 T-cell %	-0.075	-0.16	[-0.76;0.45]	0.60			
Age at nadir CD4 T-cell % [c]	0.020	0.08	[-1.11;1.27]	0.89			
Duration of CD4 T-cell % < 15 during lifetime [f]	-0.171	-0.82	[-2.20;0.55]	0.23			

Aviremic patients
Dependent variable: $CD4_{RTE}$

			Unadjusted analysis[a]			Adjusted analysis[i]		
		Median (IQR)	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
		Pearson's r						
Sociodemographic, clinical and virological variables								
Sex	Male	73 (68-77)	Reference			Reference		
	Female	79 (76-84)	6.77	[2.85;10.69]	0.001	4.85	[1.16;8.54]	0.01
Ethnicity	Black	74 (68-82)	Reference					
	Other	78 (73-80)	1.97	[3.00;6.93]	0.43			
CDC stage	Non C	77 (72-80)	Reference					
	C	75 (72-82)	0.70	[-4.18;5.58]	0.77			
HIV-1 subtype	Non B	78 (72-80)	Reference					
	B	74 (69-81)	2.41	[-3.51;8.33]	0.41			
HIV-1 coreceptor usage	R5	79 (74-80)	Reference					
	X4R5	74 (72-80)	-0.42	[-5.24;4.40]	0.86			
anti-CMV IgG	Negative	79 (76-80)	Reference			Reference		
	Positive	74 (69-80)	-3.72	[-8.07;0.62]	0.09	-0.12	[-3.;3.65]	0.99

Current status							
Age [c]	-0.161	-0.47	[-1.42;0.48]	0.33	-0.41	[-1.14;0.32]	0.26
HIV-DNA [d]	0.197	2.75	[-1.80;7.31]	0.23			
CD4 T-cell count/µl [e]	0.520	1.46	[0.66;2.26]	0.0007	1.01	[0.27;1.75]	0.009
CD4 T-cell percentage	0.370	0.38	[0.06;0.69]	0.02			
CD4/CD8 ratio	0.288	5.21	[-0.82;11.24]	0.09			
HIV disease history							
Age at first HAART [c]	-0.131	-0.28	[-0.99;0.43]	0.43			
Cumulative duration of HAART over the last 10 years [f]	0.041	0.14	[-1.01;1.29]	0.81			
Duration of last period of HIV RNA < 500 copies/ml [f]	-0.058	-0.11	[-0.77;0.54]	0.73			
Cumulative viremia over the last 10 years [g]	-0.146	-3.12	[-10.15;3.91]	0.37			
Nadir CD4 T-cell %	0.456	0.43	[0.15;0.71]	0.004			
Age at nadir CD4 T-cell % [c]	-0.055	-0.10	[-0.71;0.51]	0.74			
Duration of CD4 T-cell % < 15 during lifetime [f]	-0.446	-0.83	[-1.38;-0.27]	0.004	-0.55	[-1.03;-0.08]	0.02

[a] Median (IQR) or Pearson's r as well as results from linear regression are indicated for univariate analysis, *P* values are those from linear regression.

[b] adjusted for the variables included in the model. The following variables with *P* values < 0.20 in univariate analysis were not included in the model, because of their associations with other independent variables: HIV-1 subtype was associated with CD4 T-cell count; HIV-DNA levels and duration of the last period of HIV RNA < 500 copies/ml were associated with cumulative viremia.

[c] per year.

[d] per $\log_{10}$ HIV-DNA copies/10^6 PBMCs.

[e] per 100 cells/µl.

[f] per 10 months.

[g] per 10000 days x $\log_{10}$ HIV-RNA copies/ml of plasma.

[h] adjusted for variables included in the model. The following variables with *P* values < 0.20 in univariate analysis were not included in the model, because of their associations with other independent variables: HIV-1 subtype was associated with CD4 T-cell count; HIV-DNA levels were associated with cumulative duration of HAART over the last 10 years.

[i] adjusted for variables included in the model. The following variable with *P* values <0.20 in univariate analysis was not included in the model, because of its associations with another independent variable: nadir CD4 T-cell % was associated with duration of CD4 T-cell % < 15 during lifetime.

Table 3: Univariate and multivariate analysis of factors associated with $CD4_N$, $CD8_N$ and $CD4_{RTE}$ percentages in viremic patients

Viremic patients **Dependent variable: $CD4_N$**								
		Unadjusted analysis[a]				**Adjusted analysis[b]**		
		Median (IQR)Pearson's r	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
Sociodemographic, clinical and virological variables								
Sex	Male	57 (49-63)	Reference					
	Female	59 (54-65)	2.61	[-5.39;10.61]	0.51			
Ethnicity	Black	58 (53-63)	Reference					
	Other	60 (52-67)	3.10	[-5.07;11.26]	0.44			
CDC stage	Non C	58 (52-63)	Reference					
	C	63 (58-65)	0.21	[-9.60;10.02]	0.97			
HIV-1 subtype	Non B	60 (49-66)	Reference			Reference		
	B	58 (54-63)	1.03	[-6.95;9.00]	0.79	-1.89	[-10.32; 6.53]	0.65
HIV-1 coreceptor usage	R5	58 (52-62)	Reference					
	X4R5	64 (52-73)	5.72	[-3.12;14.56]	0.19			
anti-CMV IgG	Negative	59 (49-75)	Reference					
	Positive	58 (53-64)	-2.32	[-11.56;6.92]	0.61			

Current status							
Age [c]	-0.205	-0.92	[-2.65;0.81]	0.29	-0.81	[-2.53; 0.92]	0.34
HIV-RNA [d]	0.021	0.25	[-4.39;4.90]	0.91	1.62	[-3.07; 6.32]	0.71
HIV-DNA [e]	-0.102	-2.03	[-10.17;6.11]	0.61			
CD4 T-cell count/µl [f]	0.393	1.68	[0.13;3.24]	0.04	2.02	[0.19; 3.84]	0.03
CD4 T-cell percentage	0.475	0.48	[0.13;0.83]	0.009			
CD4/CD8 ratio	0.456	11.83	[2.72;20.94]	0.02			
HIV disease history							
Age at first HAART [c]	-0.262	-0.85	[-2.20;0.50]	0.21			
Cumulative duration of HAART over the last 10 years [g]	0.003	0.01	[-0.99;1.00]	0.99			
Cumulative viremia over the last 10 years [h]	-0.194	-5.46	[-16.34;5.42]	0.31			
Nadir CD4 T-cell %	0.248	0.38	[-0.21;0.97]	0.20			
Age at nadir CD4 T-cell % [c]	0.118	0.22	[-0.51;0.95]	0.54			
Duration of CD4 T-cell % < 15 during lifetime [g]	-0.300	-0.82	[-1.86;0.21]	0.11			

Viremic patients
Dependent variable: $CD8_N$

			Unadjusted analysis[a]			Adjusted analysis[i]		
		Median (IQR)Pearson's r	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
Sociodemographic, clinical and virological variables								
Sex	Male	20 (14-25)	Reference					
	Female	23 (18-29)	-0.01	[-7.78;7.77]	0.99			
Ethnicity	Black	21 (14-25)	Reference					
	Other	24 (16-28)	2.04	[-5.47;9.55]	0.58			
CDC stage	Non C	21 (14-27)	Reference					
	C	25 (19-29)	4.31	[-4.84;13.47]	0.34			
HIV-1 subtype	Non B	25 (18-29)	Reference					
	B	20 (14-33)	4.47	[-2.91;11.86]	0.22			
HIV-1 coreceptor usage	R5	21 (15-25)	Reference					
	X4R5	26 (16-32)	2.47	[-5.62;10.57]	0.54			
anti-CMV IgG	Negative	27 (22-36)	Reference			Reference		
	Positive	20 (14-25)	-9.24	[-16.86;-1.63]	0.02	-2.74	[-9.60;4.13]	0.42

Current status							
Age [c]	0.121	0.51	[-1.15;2.17]	0.53	0.15	[-1.09;1.38]	0.81
HIV-RNA [d]	-0.285	-3.27	[-7.63;1.08]	0.13	-1.43	[-4.66;1.79]	0.37
HIV-DNA [e]	-0.397	-7.78	[-15.19;-0.37]	0.04			
CD4 T-cell count/µl [f]	0.749	3.21	[2.09;4.33]	<0.0001	2.85	[1.51;4.18]	0.0002
CD4 T-cell percentage	0.808	0.78	[0.55;1.00]	<0.0001			
CD4/CD8 ratio	0.806	19.78	[13.92;25.65]	<0.0001			
HIV disease history							
Age at first HAART [c]	-0.032	-0.11	[-1.63;1.41]	0.88			
Cumulative duration of HAART over the last 10 years [g]	0.091	0.21	[-0.73;1.16]	0.64			
Cumulative viremia over the last 10 years [h]	-0.382	-10.76	[-21.03;-0.49]	0.04			
Nadir CD4 T-cell %	0.315	0.46	[-0.09;1.01]	0.10			
Age at nadir CD4 T-cell % [c]	-0.368	-0.62	[-1.24;-0.02]	0.05			
Duration of CD4 T-cell % < 15 during lifetime [g]	-0.190	-0.50	[-1.52;0.52]	0.32			

Viremic patients **Dependent variable: $CD4_{RTE}$**			**Unadjusted analysis[a]**			**Adjusted analysis[j]**		
		Median (IQR) Pearson's r	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
Sociodemographic, clinical and virological variables								
Sex	Male	82 (80-84)	Reference					
	Female	81 (78-90)	0.24	[-6.54;7.02]	0.94			
Ethnicity	Black	80 (77-87)	Reference					
	Other	83 (80-86)	0.03	[-7.17;7.23]	0.99			
CDC stage	Non C	81 (79-84)	Reference					
	C	84 (80-90)	2.79	[-6.14;11.71]	0.52			
HIV-1 subtype	Non B	84 (81-86)	Reference					
	B	80 (78-84)	0.96	[-5.77;7.68]	0.77			
HIV-1 coreceptor usage	R5	81 (78-90)	Reference					
	X4R5	81 (80-84)	-3.74	[-10.98;3.50]	0.29			
anti-CMV IgG	Negative	84 (81-90)	Reference					
	Positive	80 (79-84)	-0.85	[-7.99;6.28]	0.80			

Current status				
Age [c]	-0.301	-0.76	[-2.02;0.51]	0.22
HIV-RNA [d]	-0.165	-1.31	[-5.45;2.83]	0.52
HIV-DNA [e]	0.068	0.84	[-6.22;7.90]	0.80
CD4 T-cell count/µl [f]	-0.032	-0.09	[-1.65;1.46]	0.90
CD4 T-cell percentage	0.093	0.06	[-0.27;0.39]	0.71
CD4/CD8 ratio	0.289	4.62	[-3.80;13.05]	0.26
HIV disease history				
Age at first HAART [c]	-0.058	-0.11	[-1.22;1.01]	0.84
Cumulative duration of HAART over the last 10 years [g]	0.158	0.23	[-0.54;1.01]	0.53
Cumulative viremia over the last 10 years [h]	-0.064	-0.98	[-9.07;7.11]	0.80
Nadir CD4 T-cell %	0.140	0.13	[-0.36;0.63]	0.58
Age at nadir CD4 T-cell % [c]	0.285	0.32	[-0.25;0.90]	0.25
Duration of CD4 T-cell % < 15 during lifetime [g]	-0.042	-0.08	[-1.04;0.89]	0.87

[a] Median (IQR) or Pearson's r as well as results from linear regression are indicated for univariate analysis, *P* values are those from linear regression.

[b] adjusted for the variables included in the model. The following variables with *P* values < 0.20 in univariate analysis were not included in the model, because of their associations with other independent variables: nadir CD4 T-cell % and duration of CD4 T-cell % < 15 during lifetime were correlated with current CD4 T-cell count

[c] per year.

[d] per $\log_{10}$ HIV-RNA copies/ml.

[e] per $\log_{10}$ HIV-DNA copies/10^6 PBMCs.

[f] per 100 cells/µl.

[g] per 10 months.

[h] per 10000 days x $\log_{10}$ HIV-RNA copies/ml of plasma.

[i] adjusted for the variables included in the model. The following variables with *P* values < 0.20 in univariate analysis were not included in the model, because of their associations with other independent variables: age at nadir CD4 T-cell % was associated with age at the time of the study; HIV-DNA levels and cumulative viremia over the last 10 years were associated with plasma HIV-RNA.

[j] No multivariate model could be built for this dependent variable.

Figure legend

$CD4_{N,}$ $CD8_N$, and $CD4_{RTE}$ percentages, current plasma HIV RNA level and current CD4 T-cell counts. The percentages of $CD4_N$ among CD4 T lymphocytes (A), of $CD8_N$ among CD8 T lymphocytes (B) and of $CD4_{RTE}$ among $CD4_N$ T lymphocytes (C) are presented by current plasma HIV RNA detection status, as box-and-whisker plots, with the whiskers representing the minimum and maximum for all data. *P* values from the Wilcoxon test are shown. The associations between $CD4_N$ and $CD8_N$ percentages (D), $CD4_N$ and $CD4_{RTE}$ percentages (E) and $CD8_N$ and $CD4_{RTE}$ percentages (F) are shown for aviremic (open symbols) and viremic (closed symbols) patients. $CD4_N$ (G), $CD8_N$ (H) and $CD4_{RTE}$ (I) percentages are presented as a function of CD4 T-cell counts for aviremic and viremic patients. Regression lines are shown on the graphs.