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Relationship between Cell-associated HIV-1 DNA and Thymic Output in HIV-1-infected Children Initiating Antiretroviral Therapy in the PENTA 5 Trial

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Abstract (updated)

Background: Patients on ART have increases in CD4+ lymphocytes but persisting cell-associated HIV-1 DNA, even when HIV-1 RNA levels are undetectable. As thymic output is crucial to immune reconstitution in children on ART, we evaluated the relationship between HIV-1 DNA dynamics and thymic output.

Methods: Cell-associated HIV-1 DNA and T-cell receptor rearrangement excision circles (TREC), a measure of thymic output, were quantified in PBMC in 33 HIV-1-infected ART-naive children at baseline, and 4, 12, 24, 48 and 96 weeks after ART initiation (median age 7.1 years (range 0.3-15.5), CD4% 17%, HIV-1 RNA 5.0 $\log_{10}$ copies/ml). Quantification was performed by real-time PCR; copy numbers were normalised to the number of ß-actin genes, and expressed relative to 10^6 PBMC ($2x10^6$ ß-actin copies) and per ml (attributing HIV-1 DNA load to the CD4 cell fraction). Longitudinal mixed models were used to assess the effects of baseline levels and age, and changes in CD4 and HIV-1 RNA on TREC and HIV-1 DNA response.

Results: At baseline, $\log_{10}$ TREC was positively associated with CD4% and HIV-1 DNA, and inversely associated with age and HIV-1 RNA ($p<0.05$). In contrast, baseline HIV-1 DNA only depended on TREC, eg 0.82 $\log_{10}$ higher HIV-1 DNA per ml for every $\log_{10}$ higher TREC per ml ($p=0.002$); age, CD4% and HIV-1 RNA were not independent predictors ($p>0.4$). Overall, there were significant decreases in HIV-1 DNA and increases in TREC in PBMC and per ml after ART initiation ($p<0.0001$). However, decreases in HIV-1 DNA were smallest when increases in TREC were largest ($p=0.002$). There was no association between changes in HIV-1 DNA and changes in CD4% or HIV-1 RNA ($p>0.4$). The relationship between changes in HIV-1 DNA and TREC varied according to the phase of HIV-1 RNA decline ($p=0.13$). During the initial decline in RNA and periods with HIV-1 RNA stable <50 c/ml there was a smaller increase in HIV-1 DNA for every 0.5 $\log_{10}$ greater increase in TREC per ml (0.10 $\log_{10}$ copies per ml, $p=0.14$), compared with periods with HIV-1 RNA consistently >50 c/ml (0.20, $p=0.002$) and periods of transient viraemia following HIV-1 RNA suppression <50 c/ml (0.36, $p=0.01$).

Conclusions: These data suggest that overall declines in HIV-1 DNA are slowest in children with greatest increases in TREC, implying ongoing infection of naive cells. However, in children with stable HIV-1 RNA<50 c/ml, in whom TREC increases are also smaller, the infection of naive cells is occurring at a slower rate.

Background & Objectives

It is well established that in spite of strong inhibition of viral replication and increases in CD4+ lymphocytes in patients on antiretroviral therapy (ART), HIV-1 may persist in peripheral blood cells and lymphoid tissues.

As thymic output is crucial to immune reconstitution in children on ART, we evaluated the relationship between the changes in cell-associated HIV-1 DNA and changes in thymic output after initiation of ART in previously untreated children in the PENTA 5 trial[1,2,3].

PENTA 5 trial & substudy design

In the PENTA 5 trial, 128 ART-naive children were randomised to ZDV+3TC or ZDV+ABC or 3TC+ABC. 33 children (n=8, 13, 12 respectively) had sequential cellular samples available for analysis in PBMC at baseline and then at 4, 12, 24, 48, and 96 weeks after initiation of ART. Children with early disease (n=17) were also randomised to receive NFV or NFV placebo (Part A: 10 NFV, 7 NFVp): and children with more advanced disease (n=16) received open label NFV (Part B).

At baseline median age was 7.1 years, CD4% was 17% (IQR 9-24%) and mean HIV-1 RNA was 5.0 $\log_{10}$ copies/ml (SD 0.8); 4 children (12%) had already had an AIDS diagnosis before starting ART.

None of the 7 children on double NRTI only changed from dual therapy during the course of the trial, and all but one had HIV-1 RNA decline to below 400 copies/ml.

Laboratory & statistical methods

During intrathymic T-cell differentiation, progenitor cells undergo rearrangement of the T cell receptor, resulting in the formation of episomal DNA by-products, T-cell receptor rearrangement excision circles (TREC). Since TREC do not replicate with mitosis and are thus diluted with cellular division, their detection in peripheral blood cells has been proposed as a marker of thymopoietic capacity. Cell-associated HIV-1 DNA and TREC were measured in PBMC by real-time PCR, as previously described[4]. HIV-1 DNA and TREC copy numbers were normalized to the number of β-actin genes, and expressed relative to 10^6 PBMC (2 x 10^6 β-actin copies).

HIV-1 RNA levels were determined using Roche Amplicor Ultrasensitive assay (version 1.5, limit of detection 50 copies/ml). Any specimen with a result >40,000 copies/ml on the ultrasensitive assay was retested using the standard Amplicor assay.

HIV-1 DNA copy numbers are expressed

- per 10^6 PBMC
- per 10^6 CD4 cells
  - by attributing the HIV-1 DNA load to the CD4 cell fraction, given that these cells are the main target of HIV-1 infection
- per ml of blood
  - children in PENTA 5 experienced substantial increases in CD4 cells during the trial[1], so HIV-1 DNA copy numbers per cells were transformed to per ml of blood by taking into account the total number of CD4 cells per ml

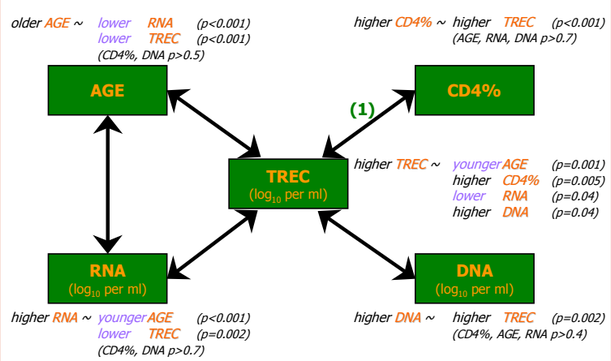
TREC per ml of blood was estimated taking into account the total number of lymphocytes per ml of blood. Longitudinal mixed models were used to assess the effects of baseline levels and age, and changes in CD4 and HIV-1 RNA on $\log_{10}$ TREC and $\log_{10}$ HIV-1 DNA after initiation of ART, replacing undetectable DNA values with half the cut-off value[5].

References (1) Paediatric European Network for Treatment of AIDS (PENTA). *Lancet* 2002; **359**:733-40. (2) De Rossi A et al on behalf of PENTA. *JID* 2002; **186**:312-20. (3) De Rossi A et al on behalf of PENTA. *AIDS* 2002; **16**:1961-1963. (4) Ometto L et al. *AIDS* 2002; **16**:839-49. (5) Hornung RW and Reed LD. *Appl Occup Environ Hyg* 1990; **5**:46-51. (7) Gibb DM et al. *Lancet* 2000; **355**:1331-1332.

Baseline HIV-1 DNA and TREC

- At baseline, children with higher TREC had higher CD4% and HIV-1 DNA; lower HIV-1 RNA; and were younger (all $p<0.05$) (Figure 1).
- In contrast, whilst children with higher HIV-1 DNA had higher TREC ($p=0.002$), age, CD4% and HIV-1 RNA were not independent predictors ($p>0.4$).
  - AIDS status at baseline did not add independent information to any relationship ($p>0.15$)
  - virtually identical results using absolute CD4 count rather than CD4 percentage
  - similarly to most studies, in a univariate analysis children with higher CD4% had lower HIV-1 RNA or were younger. However, the multivariate analysis showed that this was a result of the strong relationship between HIV-1 RNA, age and TREC, and that neither HIV-1 RNA nor age independently predicted CD4% once the TREC level was known.

Figure 1: Relationship between TREC, DNA, CD4%, RNA and age at baseline (initiation of ART)

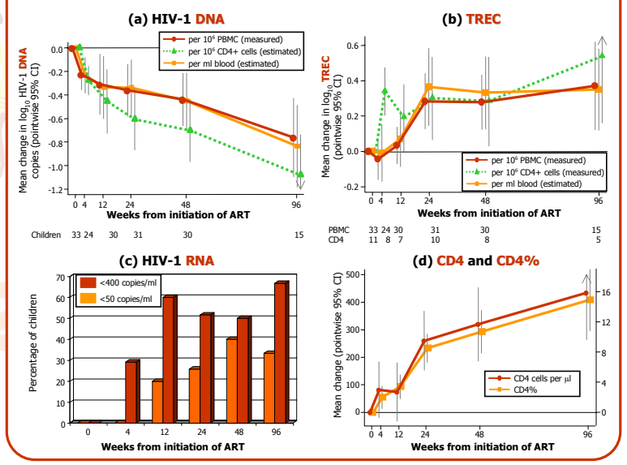


Note: arrows denote independent predictors: for example, after adjusting for TREC (arrow **(1)**), neither DNA, RNA nor AGE are independent predictors of baseline CD4% level, and these 3 factors are therefore not connected to CD4% with arrows.

Response to ART

- There was a significant decrease in HIV-1 DNA[3] and a significant increase in TREC[2] after ART (both $p<0.0001$, Figure 2(a) and (b)).
- 30 children (91%) achieved HIV-1 RNA <400 copies/ml and 19 (58%) <50 copies/ml at least once during follow-up (Figure 2(c)).
- CD4 and CD4% both increased substantially as expected (Figure 2(d)).

Figure 2: Response to ART

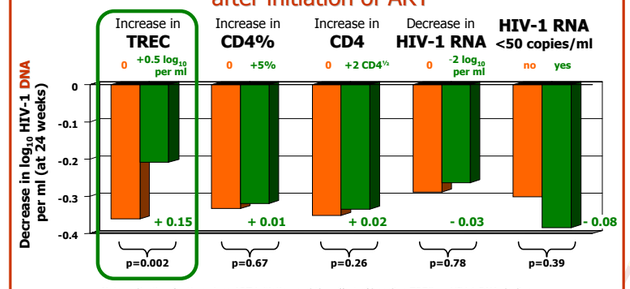


Predictors of HIV-1 DNA response to ART

We then evaluated predictors of changes in HIV-1 DNA (Figure 3).

- overall, children with the greatest increases in TREC had the smallest declines in HIV-1 DNA
  - for every 0.5 $\log_{10}$ greater increase in TREC per ml, DNA decline was 0.15 $\log_{10}$ copies per ml smaller
- changes in TREC were the strongest predictor of changes in DNA
  - changes in CD4%, absolute CD4, and RNA were not additional predictors ($p>0.4$)

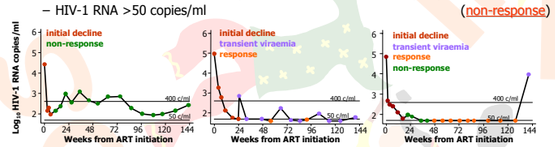
Figure 3: Predictors of changes in HIV-1 DNA after initiation of ART



Note: adjusting for time since ART initiation and the effect of baseline TREC on HIV-1 DNA decline

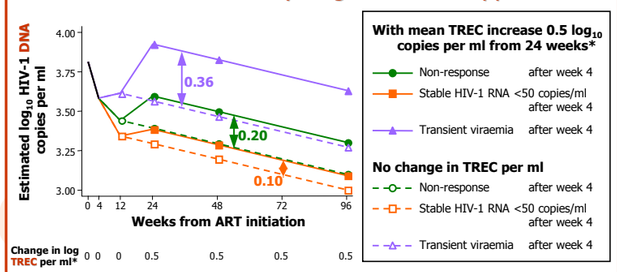
Relationship between HIV-1 DNA and TREC

- there was no significant association between changes in HIV-1 RNA and DNA
- however, maintenance of HIV-1 RNA suppression <50 copies/ml was poor in PENTA 5: only 6 children in this substudy achieved and consistently maintained full suppression, and several children experienced transient viraemia with or without subsequent persistent rebound >50 copies/ml
- we therefore classified HIV-1 RNA decline into
  - initial RNA decline to lowest point (initial decline): then after this "lowest point"
    - "stable" HIV-1 RNA <50 copies/ml (both 2 previous HIV-1 RNA<50 copies/ml) (stable response)
    - HIV-1 RNA >50 copies/ml with at least 1 of the 2 previous HIV-1 RNA<50 copies/ml (subsequent timepoints <50 copies/ml included until "stable") (transient viraemia)
    - HIV-1 RNA >50 copies/ml (non-response)



- adjusting for change in TREC and time since ART initiation, the phase of HIV-1 RNA decline was associated with change in HIV-1 DNA ($p=0.04$) – HIV-1 DNA was lower during stable response and higher during transient viraemia
  - stable response: HIV-1 DNA was 0.21 $\log_{10}$ copies/ml lower than during initial RNA decline ($p=0.05$)
  - non-response: HIV-1 DNA was 0.11 $\log_{10}$ copies/ml lower than during initial RNA decline ($p=0.53$)
  - transient viraemia: HIV-1 DNA was 0.06 $\log_{10}$ copies/ml higherthan during initial RNA decline ($p=0.20$)
  - with no change in TREC, HIV-1 DNA decline during virological response was greater by 0.10 and 0.27 $\log_{10}$ per ml than during virological non-response and transient viraemia respectively
- however, the relationship between changes in TREC and changes in HIV-1 DNA also varied according to the phase of HIV-1 RNA decline ($p=0.13$) (Figure 4) – with smaller increases in HIV-1 DNA associated with increases in TREC during stable response
  - stable response: for every 0.5 $\log_{10}$ greater increase in TREC per ml, HIV-1 DNA decline was 0.10 $\log_{10}$ copies per ml smaller ($p=0.14$)
  - non-response: for every 0.5 $\log_{10}$ greater increase in TREC per ml, HIV-1 DNA decline was 0.20 $\log_{10}$ copies per ml smaller ($p=0.002$)
  - transient viraemia: for every 0.5 $\log_{10}$ greater increase in TREC per ml, HIV-1 DNA decline was 0.36 $\log_{10}$ copies per ml smaller ($p=0.01$)
  - for each 0.5 $\log_{10}$ increase in TREC, infection of newly produced cells during virological response was lower by 0.10 and 0.26 $\log_{10}$ per ml compared to virological non-response and transient viraemia respectively

Figure 4: Estimated relationship between changes in TREC and HIV-1 DNA by stage of HIV-1 suppression



Note: decline in HIV-1 DNA estimated from a multilevel model adjusting for time since ART initiation and the effect of baseline TREC on HIV-1 DNA decline

Summary and Discussion

Persistence of HIV-1 DNA during ART may result both from memory latently infected cells with a long half-life and from newly infected cells. In children on ART, immune repopulation mainly occurs with naive CD4 CD45RA cells[7], and we have previously demonstrated that increases in naïve cells are strongly associated with increases in TREC[2,3].

Present findings indicate that

- ★ At baseline, levels of cell-associated HIV-1 DNA were higher in children with higher TREC
  - thus suggesting that total HIV-1 DNA burden is related to thymic output
- ★ Overall, during ART, HIV-1 DNA declined least at times when the increases in TREC were greatest
  - thus suggesting ongoing infection of newly thymically produced cells
- ★ The inverse relationship between decline in HIV-1 DNA and increase in TREC was stronger during non-response and transient viraemia (plasma HIV-1 RNA >50 copies/ml) than during virological response (plasma HIV-1 RNA stably <50 copies/ml)
- ★ After adjusting for changes in TREC, HIV-1 DNA decline was greater during virological response than during non-response and transient viraemia

These findings, together with the evidence that stable viral suppression was associated with smaller increases in TREC[2], suggests that:

- during non-response or transient viraemia, CD4 depletion in the periphery leads to increased thymic output; persistence of HIV-1 DNA is mainly sustained by infection of newly produced cells
- during virological response, CD4 cells survive longer in the periphery, so thymic output is not increased so much; persistence of HIV-1 DNA is mainly sustained by infected cells having a longer survival

- ★ The decline in HIV-1 DNA burden during ART depends on both HIV-1 RNA levels in plasma and changes in TREC; viral suppression in plasma and no increase in thymic output lead to the greatest decline, while transient viremia and increase in thymic output lead to the slowest decline in HIV-1 DNA burden in blood.
- ★ These findings add a cautionary note to the practice of short cycle pulse ART in children, and highlight the importance of attaining and maintaining maximal plasma HIV-1 RNA suppression.

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