HIV T-cell immunity predicts intact proviral DNA decline in people treated during acute infection

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Background

Starting antiretroviral therapy (ART) during acute HIV infection (AHI) limits HIV induced damage to the immune system and restrain the size of the viral reservoir. In the design of HIV cure interventions insight in the relation between viral reservoir and a potentially inducible immune response is important. Therefore, we investigated the viral reservoir size and HIV specific immune responses in participants of the Netherlands Cohort Study on Acute HIV Infection (NOVA study), who initiated ART immediately after diagnosis of AHI.

Methods

The NOVA study is an observational, longitudinal prospective cohort including patients with an acute HIV infection who immediately start ART (<24 hours). The NOVA study is performed in 9 HIV treating centers, shown in purple dots in Fig. 1.

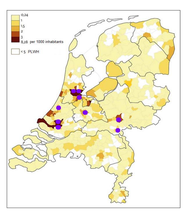


Figure 1: the Netherlands, purple dots representing where the participating HIV treating centers are located.

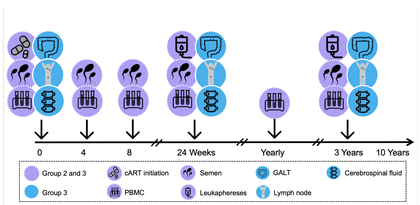


Figure 2: Sampling schedule for NOVA participants.

Acute/early infection was defined upon Fiebig stages. For our analysis, we included participants who were diagnosed during Fiebig stage II-VI. Participants can choose to participate in 3 groups (group 1=standard routine sampling). The time schedule of sampling for group 2 and group 3 is depicted in Fig. 2. In the present analysis peripheral blood samples are used obtained from leukaphereses at 24 and 156 weeks after ART initiation.

Participants in the NOVA study diagnosed during Fiebig II-VI, of which PBMC from both 24 and 156 weeks were available, were included in this analysis (n=11). Viral reservoir size was assessed by Intact Proviral DNA Assay (intact and defective proviruses), qPCR for total HIV-DNA (TD) and unspliced HIV-RNA (US-RNA) and quantitative viral outgrowth assays (QVOA). US-RNA/TD ratio represents transcriptional activity of the viral reservoir corrected for proviral DNA content. The delta (Δ) intact/defect/total provirus represents the difference between the two time points.

HIV-specific T-cell responses upon HIV peptide pool (Env, Gag, Nef, Pol) stimulation were determined as follows: activation-induced markers (AIM-assay) of HIV-specific CD4 and CD8 T cells were determined by flowcytometry for the proportion of OX40+/CD137+ CD4 T cells and CD69+/CD137+ CD8 T cells upon 1 day stimulation; IFN-γ release in the culture supernatant was determined by ELISA after 1 day stimulation; Proliferative responses and precursor frequency of CD4 and CD8 T cells were determined by flowcytometry using cell trace violet upon 6 day stimulation; Statistical analysis was performed using a paired non-parametric T test (Wilcoxon) and Pearson correlation (p<0.05).

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Results

We observed a decline in total HIV-DNA load (Fig. 3, left side, p=0.049) and the intact proviral DNA (Fig. 3, 4th graph, p=0.0078). The defective proviral DNA and cell-associated US-RNA between 24 and 156 weeks after ART initiation remained stable. Interestingly, the US-RNA/TD ratio increased over time (Fig. 3, 3rd graph, p=0.027). Isolation of replicating virus by QVOA was successful in five participants at the 24 week time point. Our data indicates that the viral reservoir has declined between 24 and 156 weeks after AHI.

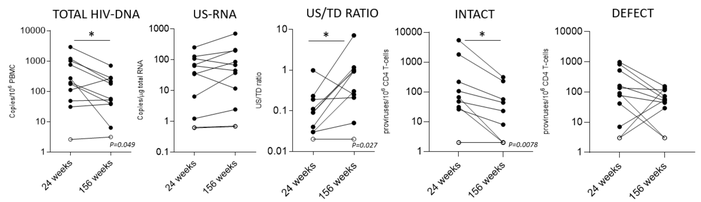


Figure 3.Viral reservoir assessments were performed by Intact Proviral DNA Assay (IPDA) and cell-associated (CA) HIV-1 RNA and total HIV-1 DNA by qPCR. The open dots represent the detection limit of the assay. A Wilcoxon rank test (paired analysis) was performed (p<0.05).

T-cell responses against Env, Gag, Nef and Pol were determined after 24 and 156 weeks. A broad HIV-specific CD4+ and CD8+ T-cell response to at least 3 different viral proteins, was observed in the majority of individuals at both time points after 1 day of peptide stimulation (AIM and IFN-γ release) (Fig. 4A,B). Overall, no significant differences in HIV-specific T cell responses were observed between the time points.

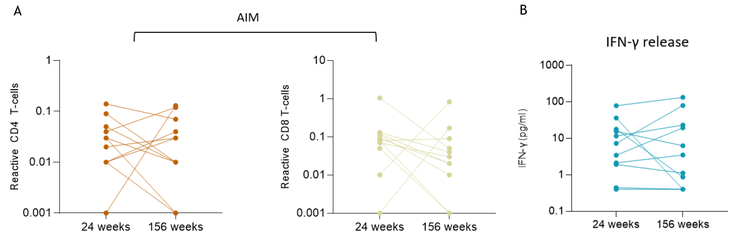


Figure 4. Determination of HIV-specific T-cell responses upon HIV-peptide stimulation (Env, Gag, Nef, Pol). **A.** Reactive CD4 and CD8 T-cells were measured after 1 day stimulation with HIV peptide pools by flowcytometry detecting activation induced markers (AIM). **B.** Interferon gamma release was measured in the culture medium after 1 day stimulation with HIV peptide pools using an ELISA.

Proliferative responses as well as the precursor frequency of HIV-specific CD4 and CD8 T-cells was determined upon 6-day HIV peptide pool stimulation. In the majority of participants a broad response of CD4 and CD8 T-cells to at least 3 HIV proteins was observed. There were no significant differences in proliferative responses (Fig.4C) and precursor frequencies (Fig. 4D) between the two time points. Our data shows that HIV-specific CD4 and CD8 T-cell responses are present early after infection (24 weeks) and sustain over time.

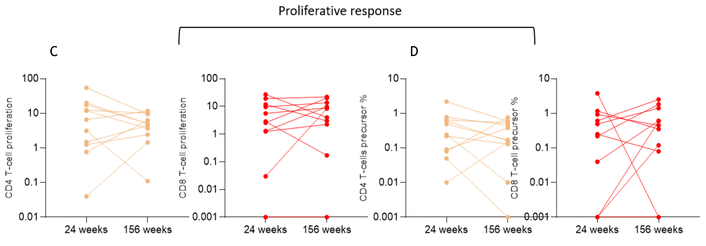


Figure 4. **C. %** HIV-specific divided CD4 and CD8 T-cells after 6 days of stimulation was measured using Cell Trace Violet. **D.** % precursors CD4 and CD8 T-cells before proliferation, measured using Cell Trace Violet.

Associations between the HIV-specific CD4 and CD8 T-cell response and the different viral reservoir markers were determined using Pearson correlation (Fig. 5&6). At 24 weeks, the intact proviral DNA load and the decay of intact proviral DNA was significantly associated with the proliferative response and precursor frequency of HIV-specific CD4+ T-cells (Fig. 5&6A,B). The decay in the total HIV-DNA load between 24 and 156 weeks was associated with the HIV-specific proliferative CD8+ T-cell responses at 24 weeks (Fig. 5&6C). At 156 weeks, intact proviral DNA load as well as the decay of intact proviral load correlated with the proliferative response and precursor frequency of CD8+ T-cells (Fig. 5&6D,E). No associations between HIV-specific T-cell responses and defective proviral DNA load and US-RNA were observed. Our data indicate that the size of the viral reservoir is directly correlated with the HIV-specific CD4 or CD8 T-cells. Interestingly, the decline of the reservoir is associated with HIV-specific proliferative responses.

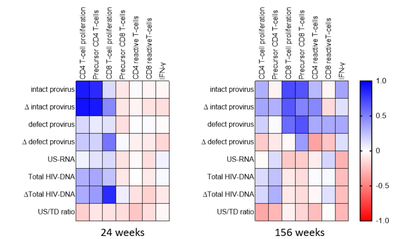


Figure 5. Correlation matrix of reservoir measurements and HIV-specific CD4 and CD8 T-cell response in the AIM and proliferation (divided cells and precursor cells) assay. The immune responses are defined as the sum of the immune responses to Env, Gag, Nef and Pol combined. Pearson correlation was used to measure the correlations (p<0.05).

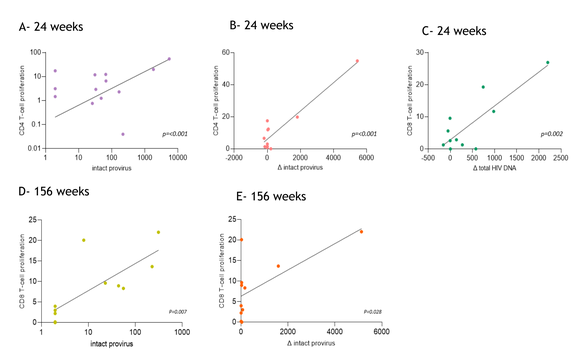


Figure 6. Correlations between several reservoir measurements and the HIV-specific T-cell responses.

Conclusions

- The proviral DNA reservoir significantly declines between 24 and 156 weeks
- We observed a positive correlation between the decay of total proviral DNA and intact proviral DNA load with the HIV-specific CD4 T-cell responses at 24 weeks and with CD8 T-cell responses at 156 weeks
- There is a trend towards a negative association between HIV-specific CD4 and CD8 T-cell responses and the US/TD ratio as measure of fraction of transcriptionally active reservoir

Taken together, these data suggest that T-cell responses specifically target the transcriptionally active reservoir. These findings potentially provide a promising avenue for cure interventions aimed at reservoir induction in combination with T-cell activating strategies.