Proportion of Inducible and Intact HIV-1 in Blood is Reflective of Lymph Nodes

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BACKGROUND

The latent reservoir (LR) for HIV-1 persists in CD4+ T cells and is a barrier to cure. The LR has been well characterized in peripheral blood, but lymph nodes have been proposed as a unique sanctuary with concerns that they harbor a greater proportion of latently infected cells, and that measurements of the LR in peripheral blood would not be representative of other tissue sites (1). Measuring the LR is complicated by an excess of defective virus that is detected by many assays but is not of concern in cure strategies. Additionally, only a subset of intact proviruses are readily activated in vitro. (2) To resolve these issues we characterized total and inducible intact and defective provirus in paired blood and lymph node samples.

METHODS

Peripheral blood and lymph node samples were collected from 8 HIV-infected, virally suppressed participants immediately prior to solid organ transplantation. Purified CD4+ T cell populations were analyzed using the intact proviral DNA assay (IPDA) to determine the number of intact and defective proviral genomes by ddPCR (3), and by a novel quantitative viral induction assay (QVIA) to determine the number of inducible intact and defective proviruses by quantification of cell-associated RNA at limiting dilution (Figure 1).

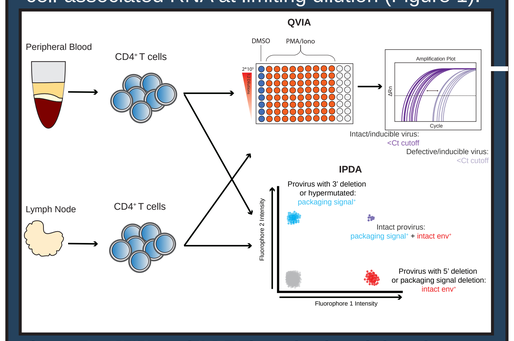


Figure 1: QVIA and IPDA in paired peripheral blood and lymph nodes samples.
Peripheral blood and lymph node samples were processed to peripheral blood mononuclear cells (PBMC) and lymph node mononuclear cells (LNMC), then CD4+ T cells were isolated. CD4+ T cells from each sample were then used for setup of IPDA and QVIA to quantify the total and inducible HIV-1 latent reservoir (LR), respectively.

RESULTS

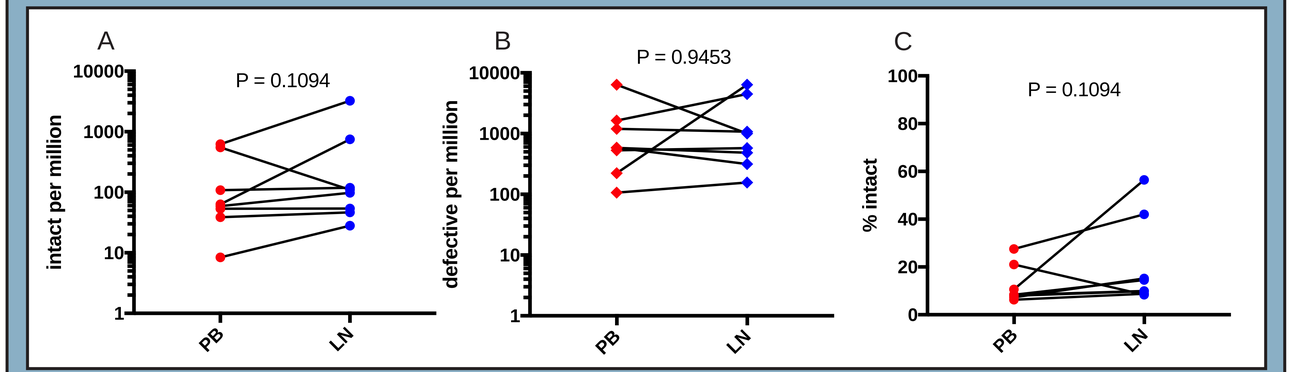


Figure 2: Total intact and defective proviral DNA measurements.
A: Intact proviruses per million cells. Lines represent paired peripheral blood (PB) and lymph node (LN) samples within each patient.
B: Defective proviruses per million cells. Each data point represents combined 5' intact or 3' intact provirus.
C: Proportion of intact over total provirus. Data are shown as % intact of total proviruses, including all intact and defective provirus measured.

RESULTS

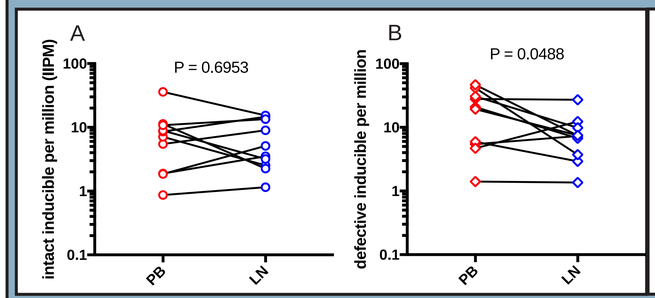


Figure 3: Inducible intact and defective HIV-1 by anatomical site.
A: Intact inducible provirus in blood and lymph node. Intact inducible virus per million cells in paired peripheral blood (PB) and lymph node (LN) samples as measured by QVIA.
B: Inducible defective virus per million cells. LN contained significantly less defective inducible virus than did PB in paired samples.

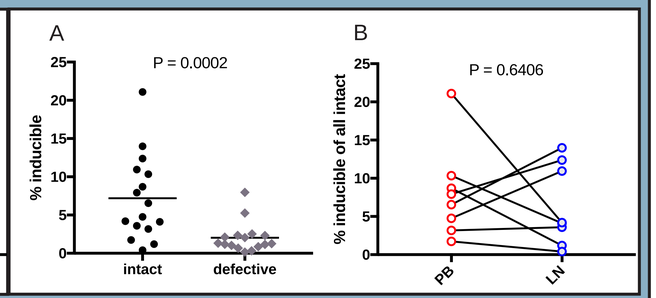


Figure 4: Proportion of inducible intact or defective provirus.
A: Inducible intact and defective HIV-1 per million CD4+ T cells in peripheral blood (PB) and lymph node (LN). Intact provirus was more likely to produce RNA than defective provirus.
B: Proportion of inducible provirus of all intact by anatomical site. No difference was detected in % inducible virus between PB and LN samples.

RESULTS

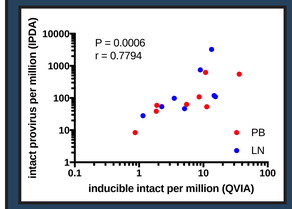


Figure 5: Correlation of total intact provirus and intact inducible provirus. Total intact as measured by IPDA correlated with intact inducible by QVIA within PB and LN samples.

The median number of intact provirus per million CD4 T+ cells was 104 (IQR 48-591) in lymph nodes and 61 (IQR=43-440) in blood; this difference did not reach statistical significance (Figure 2A). There was also no difference in defective virus per million between these sites (median=789, IQR=357-3642; median=584, IQR=299-1530; Figure 2B) The ratio of intact to total virus was median=9.9% (Figure 2C). No difference in intact inducible virus was detected between lymph node and blood samples (median= 4.3, 7.9 provirus per million, respectively; Figure 3). Only a small proportion of the intact provirus population was inducible (median=5.7%, IQR=3.3-10.8%) with an even smaller subset of inducible defective provirus (median=1.3%, IQR=0.9-2.3%) (Figure 4). Total intact by IPDA and intact inducible provirus by QVIA correlated (Figure 5).

CONCLUSIONS

Using two novel assays to analyze different properties of the HIV-1 LR we found no quantitative differences between the peripheral blood and lymph nodes. Taken together with previously reported data showing no difference in the distribution of HIV-1 proviral sequence variants between these sites (4), we conclude that levels of intact and inducible HIV-1 in blood provide a reasonable approximation of frequencies in the lymph nodes.

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