INFLAMMATORY CHEMOKINES LINKED TO HIV GENETIC DIVERSITY DURING HIV-HBV CO-INFECTION

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INTRODUCTION



Liver disease is the **third** major cause of death in HIV-positive individuals.[1]



Risk of liver disease in **co-infected** individuals is:

- **17** times higher than **HBV** mono-infected individuals[2]
- **8** times higher than **HIV** mono-infected individuals[2]



Because of these statistics, there is clearly interplay between the two viruses in the context of co-infection. Extensive work has been done on the impact of HIV co-infection on the HBV genome. However, little is understood about the impact of HBV co-infection on HIV proviruses.

AIMS

We sequenced the **HIV proviruses** in the peripheral blood of HIV-HBV co-infected and HIV mono-infected participants to determine whether co-infection itself has any impact on the HIV proviruses. We correlated **diversity of HIV proviruses** and **proportion of genetically intact HIV proviruses** with **direct and indirect clinical parameters of HBV infection** in the co-infected participants to examine any link between the two viruses.

THE PARTICIPANTS

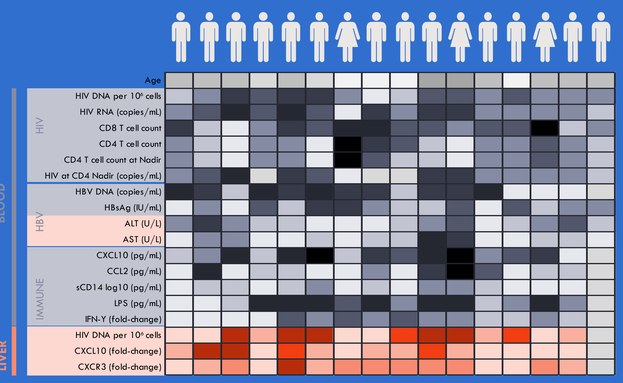
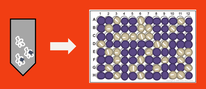


Figure 1: Clinical parameters of the 16 HIV-HBV co-infected participants obtained from our collaborators. These measurements were taken either from liver biopsies or blood as indicated on the side. Stronger colours indicate higher levels of each parameter.

16 HIV-HBV co-infected participants from Thailand. Participants were serotyped for HIV subtype AE. Whole blood draw was conducted pre-therapy.
4 HIV subtype AE mono-infected participants from Thailand. Infection parameters have not been recorded for these participants

METHODS

Full-length Individual Proviral Sequencing Assay[3]



1. Limiting Dilution: Extracted DNA was diluted until 30% of the wells have HIV



2. Nested PCR for the integrated HIV DNA using primers specific to the LTR regions to amplify ~92% of the genome[3]



3. Sequenced positive PCR reactions on the Illumina MiSeq platform and assemble and align the HIV proviruses. These HIV proviruses are then screened for defects:



Sequences without defects were termed **genetically intact**

These are the proviruses most likely to produce infectious virus. We quantify **infection frequency** of these intact proviruses per 10^6 cells and **genetic diversity** of these intact proviruses.

RESULTS AND DISCUSSION

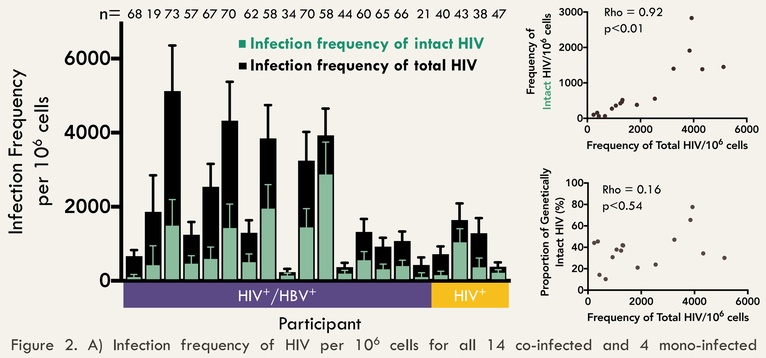


Figure 2. A) Infection frequency of HIV per 10^6 cells for all 14 co-infected and 4 mono-infected participants, where the frequency of intact HIV, in green, may also be viewed as a proportion of the total HIV frequency, in black. n denotes number of HIV sequences obtained for each participant. B) Spearman rank correlation of frequency of intact HIV and frequency of total HIV per 10^6 $CD4^+$ T cells C) Spearman rank correlation of frequency of total HIV and the proportion of sequences found to be intact HIV for each participant.

A total of 892 HIV proviruses from 16 HIV-HBV co-infected individuals and 165 proviruses from 4 HIV mono-infected individuals were analysed. Both cohorts were ART-naïve at the time of sample collection. Both the co-infected and mono-infected individuals had high levels of genetically intact HIV provirus (range = 60-3000 copies and 100-1000 copies per 10^6 CD4 T cells respectively) with no significant difference between them (Figure 2A, Figure 3A). These levels are much higher than previous studies in our group (1-10%), all conducted on participants on long-term suppressive ART[3]. Greater overall infection frequency is strongly associated with greater intact infection frequency ($p<0.0001$), but is not associated with greater proportion of intact proviruses (Figure 2B, 2C).

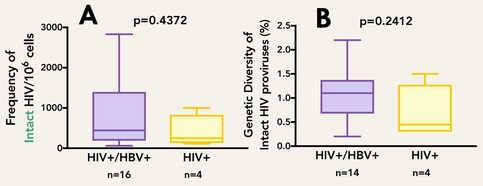


Figure 3. Comparison of the HIV proviruses between the co-infected cohort and the mono-infected cohort. A) There was no significant difference between the frequency of intact HIV between the co-infected and mono-infected cohort ($p=0.43$) B) There was no significant difference between the genetic diversity of the intact HIV proviruses between the two cohorts ($p=0.24$).

Intact sequences from the two cohorts had genetic diversity ranging from 0.2-2.2% and 0.3-1.6% for the co-infected and mono-infected cohorts respectively. The mean diversity of genetically intact provirus was lower in the HIV mono-infected (0.7%) than the HIV-HBV co-infected cohort (1.5%), but this did not reach significance (Figure 3B).

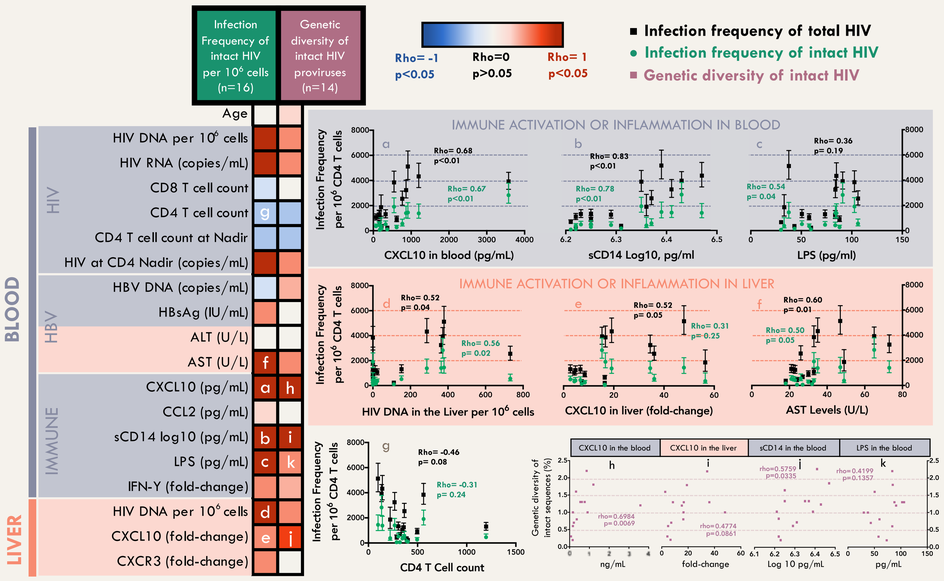


Figure 4. Correlations between viral and immunological parameters against the diversity and frequency of intact HIV proviruses in each participant. Parameters were measured in either the blood or the liver (indicated on the left hand side) and all parameters affecting the liver have been highlighted in pink. Overall data is depicted on the left as a heat map where red and blue indicate positive or negative associations respectively, and white represents no correlation (see legend). Correlations were all measured using Spearman's rank correlation. 11 correlation plots have been shown, depicting significant associations or those approaching significance: a-c) Parameters measured in the blood, relating to immune activation or inflammation, correlated with overall and intact infection frequency; d-f) Parameters either measured in the liver, or relating to inflammation of the liver, correlated with overall or intact infection frequency; g) CD4 T cell count correlated with overall and intact infection frequency, and h-k) Parameters of inflammation or immune activation correlated with genetic diversity of intact HIV proviruses.

No correlation was found between HBV viral parameters such as HBV DNA levels and the frequency or diversity of intact HIV proviruses in the co-infected individuals (Figure 4), but significant associations were found in the markers AST, CXCL10, sCD14 and LPS in the blood (Figure 4), as well as frequency of HIV in the liver. Significant correlations of AST and HIV DNA levels in the liver suggest that increased inflammation in the liver is associated with increased frequency of intact HIV. Furthermore, increased genetic diversity of HIV is also associated with CXCL10 and sCD14 levels in the blood (Figure 4). These results suggest that HBV co-infection may be worsening HIV infection through increased inflammation, although further studies need to be done to confirm this.

CONCLUSIONS

Both the HIV-HBV co-infected and HIV mono-infected participants had a very high infection frequencies for both intact and total HIV, with some participants also having a very high proportion of intact proviruses compared to defective.

No significant difference was found in the frequency or diversity of intact HIV proviruses between the two cohorts.

Increased **AST**, **CXCL10**, **sCD14** and **LPS** in the blood and **HIV DNA in the liver** were associated with frequency of intact HIV proviruses in HIV-HBV co-infected participants. Of these, **CXCL10** and **sCD14** in the blood were also associated with increased genetic diversity of intact HIV proviruses in these same participants.

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