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

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HIV AND LIVER DISEASE

• Top 3 causes of death for HIV-positive individuals:
  1. AIDS (29%)
  2. Non-AIDS Cancer (15%)
  3. Liver disease (13%)

Adapted from data by Smith, C. et al. (2014) Trends in underlying causes of death in people with HIV from 1999 to 2011
THE RELATIONSHIP BETWEEN HIV AND HBV

- 17X more likely to develop liver cancer than HBV mono-infection
- Significantly faster disease progression
- Slower clearance/more chronic HBV
THE RELATIONSHIP BETWEEN HIV AND HBV

- 8X more likely to develop liver cancer than HIV mono-infection
AIM

* Investigate the impact of HBV on HIV in vivo
PREVIOUS WORK IN THIS STUDY

PBMCs

Untreated HIV-HBV co-infected participants from Thailand

Liver

Full-length Individual Proviral Sequencing Assay
To sequence full-length HIV proviruses

~92% of the HIV proviral genome

Nested PCR for the integrated HIV DNA

PCR 1

PCR 2
PREVIOUS WORK IN THIS STUDY

PBMCs

Liver

Untreated HIV-HBV co-infected participants from Thailand

Full-length Individual Proviral Sequencing Assay
To sequence full-length HIV proviruses
**ALTERNATIVE STRATEGY**

<table>
<thead>
<tr>
<th>Age</th>
<th>HIV DNA per 10^6 cells</th>
<th>HIV RNA (copies/mL)</th>
<th>CD8 T cell count</th>
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<tbody>
<tr>
<td>BLOOD</td>
<td>HIV</td>
<td>HBV DNA (copies/mL)</td>
<td>HBsAg (IU/mL)</td>
<td>ALT (U/L)</td>
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<td>CXCL10 (pg/mL)</td>
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<tr>
<td>IMMUNE</td>
<td>HIV</td>
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<td>LIVER</td>
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<td>CXCR3 (fold-change)</td>
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</table>
DATA ANALYSIS WORKFLOW

Calculate genetic diversity

Genetically Intact HIV Provirus

Calculate infection frequency

Defective Provirus

Inversion

Large Internal Deletion

Frameshift Mutations

Point Mutation

Hypermutation

Cis-acting Mutation

Total sequenced HIV proviruses

Hiener et al. (2017) Cell Reports
ALL PARTICIPANTS HAD HIGH INFECTION FREQUENCY OF INTACT HIV PROVIRUS

Infection Frequency per 10^6 cells

n= 68 19 73 57 67 70 62 58 34 70 58 44 60 65 66 21 40 43 38 47

<table>
<thead>
<tr>
<th>Participant</th>
<th>Infection frequency of intact HIV</th>
<th>Infection frequency of total HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+/HBV+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV+</td>
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<tr>
<td>Frequency of intact HIV proviruses (n=16)</td>
<td>Genetic diversity of intact HIV proviruses (n=14)</td>
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**BLOOD**

| HIV | HIV DNA per 10^6 cells | HIV RNA (copies/mL) | CD8 T cell count | CD4 T cell count | CD4 T cell count at Nadir | HIV at CD4 Nadir (copies/mL) | HIV DNA (copies/mL) | HBsAg (IU/mL) | ALT (U/L) | AST (U/L) | CCL2 (pg/mL) | CXCL10 (pg/mL) | CXCL10 (fold-change) | IFN-γ (fold-change) | CXCR3 (fold-change) |
|-----|------------------------|---------------------|-------------------|------------------|----------------------|---------------------------|-------------------------|----------------|-----------|----------|-----------|-------------|--------------|----------------------|----------------------|----------------------|

**LIVER**

<table>
<thead>
<tr>
<th>HIV</th>
<th>HBV DNA (copies/mL)</th>
<th>LPS (pg/mL)</th>
<th>sCD14 log10 (pg/mL)</th>
<th>IFN-γ (fold-change)</th>
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**SUMMARY OF CORRELATION ANALYSES**

<table>
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<tr>
<th>Rho= -1</th>
<th>Rho=0</th>
<th>Rho= 1</th>
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<tbody>
<tr>
<td>p&lt;0.05</td>
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Spearmans rank correlation
INFLAMMATION IS ASSOCIATED WITH INCREASED FREQUENCY OF HIV

IMMUNE ACTIVATION OR INFLAMMATION IN BLOOD

- Infection frequency of total HIV
- Infection frequency of intact HIV

CXCL10 in blood (pg/mL)  
Infection Frequency per 10^6 CD4 T cells

Rho = 0.68
p < 0.01

Rho = 0.67
p < 0.01

sCD14 Log10, pg/ml

Rho = 0.83
p < 0.01

Rho = 0.78
p < 0.01

LPS (pg/ml)

Rho = 0.36
p = 0.19

Rho = 0.54
p = 0.04

Spearmans rank correlation
GREATER GENETIC DIVERSITY ASSOCIATED WITH INCREASED CXCL10 AND SCD14 IN THE BLOOD

Spearmans rank correlation
COMPARISON TO MONO-INFECTED COHORT

• No significant difference in the HIV proviruses between HIV-HBV co-infected and HIV mono-infected cohort

Mann-Whitney Test
CONCLUSION

• Higher frequency of intact HIV correlated with higher levels of AST, CXCL10, sCD14 and LPS levels in the blood
• Increased diversity of intact virus also correlated with CXCL10 and sCD14
• HIV DNA levels in the liver correlated with higher frequency of intact HIV indicating that the liver may be a site of infection and a future reservoir
• Future work will now focus on investigating the impact of HBV co-infection on proviruses after 3 years of antiretroviral therapy to see if any of these trends persist
ACKNOWLEDGEMENTS

• Thanks so much to my lab! ->
  * Bottom left to right:
    • Bonnie Hiener
    • Eunok Lee
    • Sarah Palmer
    • Beth Horsburgh
    • me
  * Middle left to right:
    • Zoe Boyer
    • Vincent Morcilla
    • Katie Fisher
    • J.S. Eden
  * Top left to right:
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    • Rory Pang
    • Jonathan Dai

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Higher frequency of HIV infection is strongly correlated with higher frequency of intact HIV but not proportion of intact HIV.

Rho = 0.92
p < 0.01

Rho = 0.16
p < 0.54
Rho = -0.31
p = 0.24

Rho = -0.46
p = 0.08
ONGOING VIRAL REPLICATION IMPLIES SAMPLING VARIABILITY

Pre-therapy participants
HIV/HBV co-infected
HIV subtype AE

On-therapy participants*
HIV mono-infected
HIV subtype B

n=878
n=1041

*Data from Horsburgh 2018
% INTACT VS % DIVERSITY

Proportion of Genetically Intact provirus (%)
## CORRELATION ANALYSES

### BLOOD

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Capsid:
Core protein
Small, medium, large surface antigens
Lipid Envelope
Positive sense DNA
Negative sense DNA covalently bound to polymerase
HBV rc genome
5' 3'
pol
LIFE CYCLE OF HBV

1. Entry through sodium taurocholate co-transporting peptide (NTCP) receptor (bile acid transporter)

2. Core-protein-mediated transportation to nucleus

3. cccDNA formation by bound viral polymerase

4. Transcription by host factors

5a. Reverse transcription of pregenomic mRNAs

5b. Translation of subgenomic mRNAs

6. Assembly and packaging

7. Viral exit via budding
1. Entry via binding CD4 and CCR5/CXCR4
2. Fusion
3. Error-prone Reverse Transcription of the RNA genome
4. Integration of provirus into host cell genome
5. Transcription, Translation and Packaging into virions