In vivo analysis of HIV replication and persistence in the myeloid compartment

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Macrophages/Microglia and HIV

- HIV-1 has evolved the ability to infect non-dividing macrophages.
- HIV-1 infection and replication in microglia/macrophages differs from that in CD4+ T lymphocytes.
- Specific cellular factors are required for HIV-1 transcription in macrophages.
- Monocytes/macrophages may serve to transmit HIV-1 to the central nervous system.
MoM: Myeloid-(only) Mouse model

• Created by transplantation of human CD34\(^+\) stem cells into pre-conditioned (irradiated) adult NOD/SCID mice.
• Human cells differentiate into B and myeloid lineages.
• However, these mice do not support human T cell production.
• Therefore the only targets for HIV infection are myeloid cells.
Experimental Design: Making MoMs

CD34+ Cells
Systemic human reconstitution in MoM

% of Live Cells

Liver | Lung | Spleen | BM
---|---|---|---
36% | 47% | 6.1% | 28%

% of human cells

CD33

Liver | Lung | Spleen | BM
---|---|---|---
44% | 47% | 6.1% | 28%

CD19

Liver | Lung | Spleen | BM
---|---|---|---
36% | 35% | 87% | 62%

CD3

Liver | Lung | Spleen | BM
---|---|---|---
<1% | <1% | <1% | <1%
Reconstitution of MoM brain with human myeloid cells
Are MoM susceptible to HIV-1 infection?
Experimental Design

• Viruses evaluated via intravenous injection
  – Typical dose contained 360,000 TCIU
  – “high” dose contained 720,000 TCIU of virus

• MoM injected with: CH040, CH040_{4013env}, JR-CSF, BaL, CH058, RHPA, HIV-2 (7321A), ADA or THRO
  – CH040_{4013env} contains a “macrophage-tropic” envelope isolated from a patient
Viruses evaluated in MoM, BLT, & TOM as demonstrated by plasma VL (& tissue vDNA)

<table>
<thead>
<tr>
<th></th>
<th>JR-CSF</th>
<th>CH040</th>
<th>CH040-4013 env</th>
<th>ADA</th>
<th>BaL</th>
<th>CH058</th>
<th>RHPA</th>
<th>THRO</th>
<th>HIV-2 (7321A)</th>
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</thead>
<tbody>
<tr>
<td>MoM</td>
<td>0/3 0/1 high</td>
<td>14/14 16/16</td>
<td>1/3 1/1 high</td>
<td>0/4 0/1 high</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>BLT</td>
<td>All</td>
<td>All</td>
<td>6/6</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>All</td>
<td>All</td>
<td>4/4</td>
</tr>
<tr>
<td>TOM</td>
<td>All</td>
<td>4/4</td>
<td>4/4</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

MoM=myeloid only humanized mouse  
BLT=bone marrow, liver, thymus humanized mouse  
TOM=T cell only humanized mice  
nd= not done
Sustained replication of virus in MoM CH040 (n=9)
Sustained replication of virus in MoM CH0404013env (n=11)
Sustained replication of virus in MoM ADA (n=2)
Lack of detectable levels of vDNA in the peripheral blood cells from MoM
Systemic presence of HIV in MoMs (tissue vDNA)
Systemic presence of HIV in MoMs (tissue vRNA)
HIV infected cells are present in MoM brain

hCD45

hCD68

HIV p24

Dr. Angela Wahl

DNA copies/10^5 cells

RNA copies/10^5 cells

4013

CH040

ADA
Rescue of replication competent HIV from tissues obtained from infected MoM

- Cells were plated and allowed to adhere overnight
- Media was aspirated and 1 million CD4\(^+\) T cells (from healthy donor) were added to the culture
- HIV-RNA levels were measured in the supernatant after 10 days

<table>
<thead>
<tr>
<th>Virus</th>
<th>Weeks Infected</th>
<th>ART?</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
<th>BM</th>
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</thead>
<tbody>
<tr>
<td>ADA</td>
<td>6</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CH040</td>
<td>4</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CH040-4013</td>
<td>5</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CH040-4013</td>
<td>5</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
HIV infection of MoM cells

- MoM are systemically reconstituted with human myeloid and B cells (absence of T cells)
- MoM can systemically replicate HIV-1 over time → Only certain viruses (CH040, CH040-4013, and ADA)
- Replication competent virus can be isolated from all infected MoM tissues
ART regimen

• Raltegravir – integrase inhibitor
• Tenofovir – NRTI
• Emtricitabine – NRTI

Mice administered treatment daily i.p.
ART effectively suppressed VL in CH040 infected MoMs
ART effectively suppressed VL in CH040\textsubscript{4013env} infected MoMs
Viral load is effectively suppressed using ART in HIV infected MoM
Differences in suppression kinetics between MoMs, ToMs and BLTs

<table>
<thead>
<tr>
<th>Hu Type</th>
<th>Virus</th>
<th>Weeks to undetectable plasma viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoM</td>
<td>CH040-4013 env</td>
<td>1-2 weeks</td>
</tr>
<tr>
<td>BLT</td>
<td>CH040</td>
<td>3-6 weeks</td>
</tr>
<tr>
<td>ToM</td>
<td>CH040</td>
<td>2-7 weeks</td>
</tr>
<tr>
<td>MoM</td>
<td>CH040</td>
<td>1-3 weeks</td>
</tr>
<tr>
<td>BLT</td>
<td>CH040</td>
<td>3-7 weeks</td>
</tr>
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</table>
Summary

• MoM are systemically reconstituted with human myeloid cells.
• MoM infection with HIV results in sustained levels of viral RNA in PB and tissues in the complete absence of T cells demonstrating the direct contribution of myeloid cells to HIV replication \textit{in vivo}.
• HIV replication in MoM is efficiently suppressed by ART.
• In MoM myeloid cells repopulate the brain.
• Myeloid cell in the brains of MoM are susceptible to productive HIV infection.
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Questions?