Macrophages in Brain: a cellular Reservoir of SIV

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HIV Persistence in Tissues & Cells

Overview – What we know:

• CD4+ T cells in blood are persistently & latently infected during cART.

• CD4+ T cells in lymphoid tissues, including spleen are likely to be persistently and a source of virus.

• Extremely low level of monocytes in blood that contain evidence of virus.

• Macrophages in tissues: lymph nodes, spleen, heart, lung, brain, liver and gut are infected.
HIV Persistence in Tissues & Cells

Overview – Unknown:

- Macrophages in tissues unclear whether there is persistence or latency and the longevity of these cells in tissues.

- Microglia, the resident brain macrophage, resides life-long, is infected and presence of HIV in CSF during cART suggests virus persistence.

- Unclear whether macrophages in lung and spleen are persistently infected or how long the different populations reside in tissue.

- Other cells? Astrocytes in brain, most abundant cell estimated 8% are HIV infected. Persistence??
HIV/SIV Macrophage Infection

- All lentiviruses infect monocytes & macrophages.

- Macrophages involved in HIV/SIV:
  - Brain – encephalitis and cognitive disorders
  - Peripheral nerves - Peripheral Neuropathy
  - Lung – pneumonia
  - Heart - Cardiomyopathy

- Infected macrophages persist for months; potential of cell to cell virus spread.

- Microglia established early in development and remain lifelong (Ginhoux et al Science, 2010 ….).

- Microglial cells comprise 10-20% of brain cells. Microglia & perivascular macrophages infected in HIV and SIV infected brain.

- Macrophages are potential reservoirs for latent HIV.
SIV Macaque Model AIDS & CNS Disease

- Infection with macrophage-tropic, neurovirulent virus & immunosuppressive virus swarm.
- Rapid, consistent AIDS in 84 d & 90% CNS disease.
# Macrophages & Microglia - SIV infection in Brain

<table>
<thead>
<tr>
<th>Selection</th>
<th>Time p.i.</th>
<th>SIV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perivascular m(\phi)</td>
<td>4 days</td>
<td>150</td>
</tr>
<tr>
<td>Microglia</td>
<td>4 days</td>
<td>200</td>
</tr>
<tr>
<td>Perivascular m(\phi)</td>
<td>84 days</td>
<td>(2.6 \times 10^7)</td>
</tr>
<tr>
<td>Microglia</td>
<td>84 days</td>
<td>(1.8 \times 10^8)</td>
</tr>
</tbody>
</table>

* Copy equivalents/\(\mu\)g RNA

Perivascular macrophages (CD14\(^+\)) & microglia (CD14\(^-\) \& CD11b\(^+\)) infected acute and late stages of disease.
Quantitation of SIV Infected Macrophages in Spleen

CD68\(^{-}\) cells + SIV RNA

4d 1 infected macrophage/100 infected cells

84d 1 infected macrophage/10 infected cells

CD68\(^{+}\) Macrophages + SIV RNA

Spleen a potential large reservoir of infected CD4+ T cells & macrophages
SIV Macaque Model of cART

Dinoso et al., JV, 2009

Untreated

cART

SIV RNA copy eq./ml

Days post-inoculation

No Tx

HAART

Pd12

Pui2

Pez1

Pyd2

Psk2

Pei2

Pfy1

Poy1

0 12 180

Days p.i.

Tenofovir, Atazanavir, Saquinavir, Integrase Inhibitor

X

Towards an HIV Cure

People focused science driven

International AIDS Society

Stronger Together

Dinso et al., JV, 2009
Latently Infected SIV Resting CD4+ T cells

Plasma

Tissues

Dinoso et al., JV, 2009
### Residual SIV RNA Replication in SIV Macaques on cART

<table>
<thead>
<tr>
<th>Tissue</th>
<th>cART</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph Node</td>
<td>20 - 165 copies /ug RNA</td>
<td>$10^7$ copies /ug RNA</td>
</tr>
<tr>
<td>Spleen</td>
<td>20 - 100 copies /ug RNA</td>
<td>$10^{7-8}$ copies /ug RNA</td>
</tr>
<tr>
<td>Lung</td>
<td>~ 10 copies /ug RNA</td>
<td>$10^6$ copies /ug RNA</td>
</tr>
</tbody>
</table>
Persistent SIV Replication with cART in Spleen

3 cells/100 cells in spleen contain SIV DNA
cART Suppresses CSF Virus & CNS Disease: Viral DNA Reservoir

Low residual levels of viral RNA in brain
NO difference in viral DNA

Zink et al., JID, 2010
In Situ Hybridization in SIV macaque Brain: cART
Microglia or Macrophage Latency

- Isolated microglia brain, cultured with CEMX174 cells.
- Activation by TNF$_\alpha$: increased virus recovered 5-100 fold.
- Cultured for 14 days, p27 and RT-PCR on supernatant & cells.
- RT-PCR in supernatant reflected virus.

Assay based on Shen et al JVI, 2007
Quantitation of SIV-infected Microglia

<table>
<thead>
<tr>
<th>Microglia</th>
<th>SIV-Infected</th>
<th>CNS Severity</th>
<th>SIV HAART</th>
<th>Suppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/100</td>
<td>X X</td>
<td>Severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1000</td>
<td>X</td>
<td>Severe</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>X</td>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/5000</td>
<td></td>
<td></td>
<td>X</td>
<td>YES</td>
</tr>
<tr>
<td>1/10000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/50,000</td>
<td></td>
<td></td>
<td>X</td>
<td>YES</td>
</tr>
<tr>
<td>1/100,000</td>
<td></td>
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Conclusions

• SIV macaque model: significant infection in macrophages in brain and spleen.

• SIV macaque cART: persistent replication in tissue despite suppressed virus in plasma and CSF.

• Quantitation of microglia in brain by limiting dilution assay demonstrates SIV latency and recovery of replication competent virus.

• Comparable limiting dilution assay should be used to examine HIV latency in tissue macrophages.
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