Effects of HIV and Aging on the brain

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Cognitive impairment despite cART
Hutton et al., 2011
n=1555 (CHARTER cohort)
50% with mild cognitive impairments
No correlation with CSF TNF, MCP-1 or viral load

<table>
<thead>
<tr>
<th>CD4 nadir</th>
<th>VL in plasma</th>
<th>HAND</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;200</td>
<td>detectable</td>
<td>45%</td>
</tr>
<tr>
<td>&lt;200</td>
<td>all</td>
<td>50%</td>
</tr>
</tbody>
</table>

Raltegravir Treatment Intensiﬁcation Does Not Alter Cerebrospinal Fluid HIV-1 Infection or Immunoactivation in Subjects on Suppressive Therapy

HIV dementia and aging-CDC data 1987-1991:
(Janssen, et al., Neurology 1992)

n=1555 (CHARTER cohort)
50% with mild cognitive impairments
No correlation with CSF TNF, MCP-1 or viral load

CD4 nadir | VL in plasma | HAND |
-----------|--------------|------|
>200       | detectable   | 45%  |
<200       | all          | 50%  |

Heaton et al., 2011
Cognitive impairment despite cART
J Am Geriatric Soc 2012

J Acquir Immune Defic Syndr 2012

Effect of Aging and Human Immunodeﬁciency Virus Infection on Cognitive Abilities

Synergistic Effects of HIV Infection and Other Age-Related Functioning

HADL Severity
Increased cognitive impairment despite anti-retrovirals

Heaton et al., 2011; CHARTER cohort; n=1555

Vivithanaporn et al., 2010

Relationship of Age To Markers of Neurocognitive Decline in CSF

Ranki et al., 1995

Latent Infection in astrocytes

Productive infection in perivascular macrophages

Tat

Jones et al., 2000; Kruman et al., 1998

Dentate Gyrus

normal

HIV no encephalitis

HIVE

HIVE + drug abuse

Normal

HIVD

Jones, Bell and Nath (unpublished)

Courtesy: Chris Power, U. Alberta
Amyloid plaques are more frequent in HIV cases

Esiri et al., J Neurol Neurosurg Psychiatry 1998

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Gray: HIV+ cases</th>
<th>White: HIV-controls</th>
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<tbody>
<tr>
<td>30-39</td>
<td>20%</td>
<td>10%</td>
</tr>
<tr>
<td>40-49</td>
<td>30%</td>
<td>20%</td>
</tr>
<tr>
<td>60-69</td>
<td>40%</td>
<td>30%</td>
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</table>

Amyloid deposition and duration of HIV infection

(Rempel and Pulliam AIDS 2005)

Control HIV Alz Demen

Accelerated Aβ deposition in APP/PS1/gp120 mice

Detection of Tat in Brain and CSF despite adequate antiretroviral therapy

Johnson et al., PNAS 2013
Tat causes neuronal injury and glial cell activation

Magnuson et al., Ann Neurol 1995

Nath et al., JBC 1999

Synergistic neurotoxicity of Tat with gp120 or glutamate (Nath et al., 2000)
Tat and amyloid beta peptide form plaques that are neurotoxic

Hategan et al., Nature Struc Mol Biol 2017

Hategan et al., Nature Struc Mol Biol 2017

Pathophysiology

Amyloid deposition
Genetic factors
ApoE
Chronic Inflammation
Neuronal injury
ApoE4 is associated with dementia in older but not younger HIV+ individuals

<table>
<thead>
<tr>
<th>APOE4 allele</th>
<th>Age group</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Younger HAD</td>
<td>No HAD</td>
<td>Older HAD</td>
<td>No HAD</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>No</td>
<td>10 (83.3)</td>
<td>15 (69.0)</td>
<td>57 (79.1)</td>
<td>72</td>
</tr>
<tr>
<td>Yes</td>
<td>3 (16.7)</td>
<td>25 (34.2)</td>
<td>15 (20.8)</td>
<td>72</td>
</tr>
</tbody>
</table>

(Jacour, et al., J NeuroImmunology 2004)

ApoE4, oxidative stress and HIV dementia

(Tat in macrophages in HIVE and SHIVE)

Select Neuronal Vulnerability with Tat

(Jones et al., 2000; Kneman et al., 1998)
11/18/17

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>No Treatment</th>
<th>Beads Alone</th>
<th>+ HIV (Mid)</th>
<th>+4900008</th>
<th>+4900035</th>
<th>+4900043</th>
<th>+4900037</th>
<th>+4900026</th>
<th>+4900029</th>
<th>+4900040</th>
<th>+4900004</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/‐</td>
<td>+</td>
</tr>
<tr>
<td># of Events</td>
<td>% Gated</td>
<td>% Total</td>
<td># of Events</td>
<td>% Gated</td>
<td>% Total</td>
<td># of Events</td>
<td>% Gated</td>
<td>% Total</td>
<td># of Events</td>
<td>% Gated</td>
<td>% Total</td>
<td># of Events</td>
</tr>
<tr>
<td>No Treatment</td>
<td>-</td>
<td>22 events</td>
<td>0.22%</td>
<td>0.22%</td>
<td>Beads Alone</td>
<td>-</td>
<td>20 events</td>
<td>0.20%</td>
<td>0.20%</td>
<td>+ HIV (Mid)</td>
<td>+</td>
<td>241 events</td>
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</table>
**Therapeutic Strategies against HIV-Tat protein**

- Block effect of Tat on LTR to prevent HIV replication
- Block neurotoxic effects of Tat

**CNS ESCAPE**

Lisa Henderson, Bryan Smith; unpublished

**Limit of detection**

Fateh Kashanchi GMU; unpublished

**COMPARISON BETWEEN PATIENT SAMPLES AND NEGATIVE CONTROLS**

Therapeutic Strategies against HIV-Tat protein

- Block effect of Tat on LTR to prevent HIV replication
- Block neurotoxic effects of Tat
TAT ANTISENSE OLIGONUCLEOTIDES

ASO directed against Tat mRNA diminish Tat production

Human astrocytes:

ASO Efficacy in lymphocytes

HIV-1 Tat increases neuronal Aβ generation.

Lisa Henderson; unpublished
Atomic Force Microscopy (AFM)

- nm resolution on biological samples

Protein distributions on the surfaces of viruses: detail of capsids of mimivirus and cyanophage. (Kuznetsov & McPcPherson, 2011)

HIV-Tat B protein in PBS

from monomers to large oligomers

Amyloid β fibrils

most frequent structure
uniform along length

200 µM amyloid A

Dimensional analysis of fibrils

increase in distance between twists due to Tat

increased rigidity of fibrils
Neurotoxicity of amyloid β Tat B fibrils

What drives T cell activation in patients on HAART?

Other Infections: E.coli – LPS; CMV
Abnormal Innate and cellular immune responses
HIV

HIV-1 LTR Truncation Mutants

Development of a High Throughput Assay for Tat inhibitors

Richa Tyagi and Wenxue Li; unpublished

Nicholas Geiger; unpublished
What is in the pipeline: Strategies against HIV-Tat protein

Assays to screen for pharmacological agents

- LTR Luc
- LTR-D1 Luc
- LTR-D2 Luc
- LTR-D3 Luc

Wenxue Li; Richa Tyagi

HIV-1 LTR Truncation Mutants

In vitro model of HIV-1 LTR transactivation

- Induction
- Production of Tat & GFP
- LTR Transactivation
- Luciferase Expression

Nicholas Geiger; unpublished

Screening of TRE3G-Tat-coGFP

Nicholas Geiger; unpublished
**Screening data-Summary**

- 2000 compounds screened in triplicate.
- Many compounds were toxic and decreased GFP.
- 105 compounds blocked Tat-dependent LTR activation and had no toxic effects on GFP expression and Tat production.
- Not known how many compounds may inhibit mLuciferase directly.

**HIV Tat binding compounds**

- **Biacore T200 Binding Constant**
  - | Compound | Ka (1/Ms) | kd (1/s) | K_D (M) |
  - | 1 | 0.86 | 6.804E-4 | 8.613E-7 |
  - | 2 | 0.198 |
  - | 3 | 1.25 |
  - | 4 | 4.5 |

**Protection from Tat toxicity**

- **Compound Assay Flowchart**
  - 1) Primary screen Tat dependent LTR activation
  - 2) Secondary Tat LTR Assay
  - 3) Orthogonal screen with HIV replication
  - 4) Direct interaction with Tat-ITC
  - 5) Protection from HIV Tat toxicity in neuronal cultures
Summary

- There is acceleration of neurocognitive deficits with aging.
- Patients with APOE4 are more vulnerable to these effects.
- There is uncontrolled Tat production and deposition of amyloid in brain despite ART.
- We have developed an antisense approach and a high throughput assay for developing Tat antagonists.
Future Directions

• Determine mechanisms of Tat induced glial cell activation

• Determine mechanism of Tat-amyloid complex induced neurotoxicity

• Identify compounds that block Tat effects on HIV replication and neurotoxicity

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Joseph Steiner
Nicholas Geiger