Sialoadhesin Expression on Monocytes Enhances HIV-1 Infectivity

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April 16, 2007

Funded by NIMH
Why we use the M/MØ to study/predict HAD?

- Activation state of the M/MØ may predict risk of HAD
- Activation state of the M/MØ gives us an idea of the subjects response to HIV infection or therapy
- M/MØ traffic into the brain - Kim et al 2005, 2006
- M/MØ subsets (CD14/CD69) and soluble factors correlate with HAD - Pulliam 2004
Using gene microarrays to characterize monocyte activation
Whole blood (CPT tube) → centrifugation → Serum, Percoll, RBC → Percoll gradient enrichment → PBMC → Magnetic beads purification → CD14+ monocytes → RNA isolation → Raw data → Data processing → Amplification and labeling → Codelink Hybridization → Array scanning
Gene Ontology
HVL vs LVL
(confirmed by RT-PCR and IHC)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Fold change</th>
<th>p</th>
<th>Brief description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotaxis genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>4.2</td>
<td>&lt; 0.01</td>
<td>chemokine ligand 2</td>
</tr>
<tr>
<td>CCR5</td>
<td>2.5</td>
<td>&lt; 0.01</td>
<td>chemokine receptor 5</td>
</tr>
<tr>
<td>Response to stress genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN (CD169)</td>
<td>3.8</td>
<td>&lt; 0.01</td>
<td>sialoadhesin</td>
</tr>
<tr>
<td>Inflammatory response genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>4.2</td>
<td>&lt; 0.01</td>
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<tr>
<td>CCR5</td>
<td>2.5</td>
<td>&lt; 0.01</td>
<td>chemokine receptor 5</td>
</tr>
<tr>
<td>CD16</td>
<td>1.5</td>
<td>0.07</td>
<td>receptor for Fc fragment of IgG</td>
</tr>
<tr>
<td>Interferon-induced genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 genes</td>
<td>31.2</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Pulliam, Sun, Rempel, J Neuroimmunology 2004 (13 HVL/10 LVL)
### Hybrid monocyte/macrophage phenotype in HVL HIV infection
(determined by gene microarrays)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Monocyte</th>
<th>Macrophage</th>
<th>HIV mono</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD16</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCR5</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCP-1</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sn (CD169)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>TNFα</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Pulliam, Sun, Rempel, *J Neuroimmunology* 2004
What does sialoadhesin do and what, if any, is the affect on HIV infection?
Sialoadhesin (Sn)/CD169

- Sn is the prototypic member of the Siglec family
- Sialic acid binding receptor (not phagocytic) present on subsets of macrophages (BM, spleen, lymph nodes), not on monocytes or microglia
- Associated with adhesion, chemotaxis and chronic inflammation
- Sn expression found on infiltrating macrophages in rheumatoid arthritis  Hartnell 2001
- Stimulated by glucocorticoids, TNFα and IFNδ in vitro Crocker 2001
- Sn-expressing macrophages found associated with AIDS-KS biopsies Cornelissen 2003
- Sn elevated in early HIV-1 infection despite HAART van der Kuyl 2007
Protein structure of Sn

C2-set region

sialic acid binding

cell membrane
Sn Expression
Patient Data

• CD14+ monocytes bead isolated
• 15 LVL (<10,000); all on HAART
• 10 HVL (>10,000); all on HAART
• 5 HIV seronegative controls
• Flow cytometry with mAb to Sn binding region
Sn expression correlates with viral load

**p < 0.001
*p < 0.02

x = 175
x = 253
x = 427

**p < 0.001
*p < 0.02
Sn expression *in vitro*

Sn-expressing cell line

- Cloned the Sn gene by PCR
- Transduced THP-1 cell line
- Clonal selection of Sn-expressing cells
- Isolated high Sn expresser
- Produced stable Sn-expressing THP-1 cells (TSn)
Sn expressed on THP-1 cells (TSn)

Western blot

Flow cytometry

TSn  THP-1  Mono  HVL  Mono control  Size Marker

kDa  

Cells

Intensity
What induces Sn expression *in vivo*?

- IFN-$\gamma$ and TNF-$\alpha$ induce Sn expression on macrophages *in vitro* - Hartnell et al, 2001

- Microarray data identified IFN - induced genes in monocytes from subjects with HVL - Pulliam et al, 2004
**Interferon-induced genes**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold LVL(^a) vs C(^b)</th>
<th>Fold HVL(^c) vs LVL(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interferon-(\alpha) induced</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFI27</td>
<td>3.9</td>
<td>166.5**</td>
</tr>
<tr>
<td>MX1</td>
<td>1.5</td>
<td>5.3**</td>
</tr>
<tr>
<td>IFIT3</td>
<td>1.4</td>
<td>4.8**</td>
</tr>
<tr>
<td>IFI44</td>
<td>1.8</td>
<td>3.7**</td>
</tr>
<tr>
<td>IFIH1</td>
<td>1.0</td>
<td>3.0**</td>
</tr>
<tr>
<td>G1P3</td>
<td>1.7*</td>
<td>2.4**</td>
</tr>
<tr>
<td>ISGF3G</td>
<td>1.4</td>
<td>1.5*</td>
</tr>
<tr>
<td><strong>Interferon-(\gamma) induced</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBP1</td>
<td>1.8</td>
<td>2.2*</td>
</tr>
<tr>
<td>IFI16</td>
<td>1.1</td>
<td>1.8**</td>
</tr>
<tr>
<td>GBP2</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>IFI30</td>
<td>1.0</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

\(\text{**}\) \(p<0.01\), \(\text{*}\) \(p<0.05\)

\(^a\) LVL - low viral load (<10,000 RNA copies per ml)
\(^b\) HVL - high viral load (\(\geq\)10,000 RNA copies per ml)
\(^c\) C - HIV seronegative control
IFN-\(\alpha\) induces Sn expression
Do Sn and HIV-1 interact?

Sialic acid binding

Sn

Monocyte cell membrane
Does HIV-1 bind to Sn?

Protocol

• Replication deficient HIV-1, deletion in TAT-REV, (NIH AIDS Research and Reagent Program)

• Primary monocytes and IFN-induced monocytes (500 IU IFN-α) were incubated with HIV-1Δ INXS for 1 h at 37°C

• Following repeated washings to remove non-specific bound HIV-1Δ, cells were lysed and assayed for p24

• Binding specificity was evaluated using an anti-Sn mAb that blocks Sn N-terminus and prevents HIV-1 binding

• And pre-treated HIV-1Δ with sialidase, to remove sialic acid conjugates from gp120
IFN-α-induces HIV-1 binding

- IFN-α induces HIV-1 binding.
- The figure shows the p24 levels (pg/ml) in different treatments.
  - HIV-1: Monocytes and IFN-induced Sn+ monocytes.
  - Sn antibody and sialidase pretreatment conditions.

The bar chart illustrates the comparison of p24 levels under various conditions, highlighting the impact of IFN-α on HIV-1 binding.
Summary

• Sn expression correlates with viral load

• Sn expression extended to CD14\(^+\) monocytes

• Binding requires cellular expression of Sn and sialic acid conjugates on the virus

• IFN-\(\alpha\) up-regulates Sn expression on monocytes, which then binds HIV-1
Can Sn-associated HIV-1 infect target cells \textit{in trans}?

- HIV - 1 = lab-adapted virus NL4-3
- Target cells = HeLa cells transfected with constitutive expression of CD4, CXCR4 and CCR5 \textit{And} co-transfected with reporter gene luciferase (relative light units) under the Tat promoter (TZM-bl)
Can Sn-bound HIV-1 *Trans* Infect?

**Monocytes**

<table>
<thead>
<tr>
<th>Test tube</th>
<th>HIV bound – Sn+ monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x washing</td>
<td>3x washing</td>
</tr>
</tbody>
</table>

**TZM-bl cells**

- **No infection**
- **Trans infection**
Results: Trans infection

**Monocytic cell line**

- THP-1
- TSn
- TSn + mAb

**Human monocytes**

- mono
- mono / IFN

Relative Light Units (RLU)
Potential sources of HIV-1

Trans

Budding virus from infected monocytes blocked by PI (Indinovir)
Confirms transinfection: monocytes cannot produce infective HIV-1 NL4-3 in the presence of indinavir
Can Sn Enhance HIV-1 Infectivity?

Sn+ monocytes  Free HIV

HIV bound - Sn+ monocytes

Relative Light Units

Trans infection

TZM-bl cells  Enhanced infectivity
Sn Enhances HIV-1 Infection

Monocytic cell line

Human monocytes

Relative Light Units (RLU)
Does Sn-bound HIV (NL4-3) use predicted receptors?

- mAb CD4 blocks CD4
- mAb CCR5 blocks CCR5
- TAK779 blocks CCR5
- mAb CXCR4 blocks CXCR4
- AMD3100 blocks CXCR4

HIV-1 Infection (Relative Light Units)
Summary

• HIV (NL4-3) bound to TSn cells can infect susceptible cells (*Trans infection*)

• *Trans* infection does not require monocyte infection

• HIV bound to Sn (IFN-\(\alpha\)-induced) on human monocytes trans infects reporter cells

• Monocytes expressing Sn capture free HIV and *trans* infect reporter cells

• Sn expression *enhances* HIV infectivity over 5-fold compared to free virus
What are the implications for CNS infection?
Single label immunohistochemistry for Sn in frontal cortex sections. (A) seronegative control, (B) HIV-1, (C) HAD and (D) HIVE.
Potential impact on CNS Infectivity

- HIV bound to Sn may be transported to the CNS during normal monocyte/macrophage trafficking
- Sn may facilitate \textit{trans} infection of susceptible cells in the CNS
- Sn may facilitate/enhance HIV infection in subjects with LVL - possibly explaining why they continue to have CNS infection
- Sn-expressing MØ cause myelin degeneration and axonopathic changes in PLP transgenic mice \textit{Ip et al 2007}
Sn causes demyelination
I. Kobar et al, Mol Cell Neurosci 31: 685 2006

Used a mouse model for inherited demyelinating neuropathy

CD8 T cells and macs influenced demyelination

Sn involved in mac-T cell interaction

Sn binds activated T cells
Possible mechanism for CNS infection

Markers
MCP-1
CCR5
CD16
Sn

High Viral Load
(IFN-α)
monocytes → Sn+ monocyte

Sn-bound HIV

Trans infection of T cell

Transmigration

BBB

CNS

Infected cells

Trans infection
Acknowledgements

Hans Rempel, PhD
Bing Sun, MD, PhD
Cyrus Calosing, BS
CD16 expression

CD16\(^+\) subset of Sn\(^+\) monocytes