SERINCs: novel restriction factors counteracted by HIV Nef

Heinrich Göttlinger, MD
Molecular, Cell and Cancer Biology
UMass Medical School
HIV-1 Genes and Proteins

HIV-1

HIV-2
Intrinsic cellular-defense against retroviruses

- Nuclear import
- Reverse transcription
- Capsid Restriction Factors
- Nuclear import and integration

- Envelope protein
- Cytidine Deaminases
- Vif
- Tetherin
- SAMHD1
- Assembly
- Vpx
- Vpu
Role of HIV-1 Nef

• Crucial for high viral loads and disease progression

• In cell culture:
  – Robustly down-regulates CD4
  – Down-regulates MHC-I
  – **Enhances infectivity**
    • CD4-independent
    • No obvious effect on virion
    • Producer cell-dependent
    • Effect depends on **dynamin, clathrin, AP2**
    • Effect mimicked by **MLV glycoGag**
    • Effect determined by **Env**
HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation

Annachiara Rosa¹*, Ajit Chandel¹*, Serena Ziglio¹*, Veronica De Sanctis², Roberto Bertorelli², Shih Lin Goh³, Sean M. McCauley³, Anetta Nowosielska³, Stylianos E. Antonarakis⁴,⁵, Jeremy Luban³, Federico Andrea Santoni⁴ & Massimo Pizzato¹

SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef

Yoshiko Usami¹*, Yuanfei Wu¹* & Heinrich G. Göttlinger¹
MLV glycoGag has Nef-like effect on HIV infectivity

5’ end of murine leukemia virus:

LTR

CTG  ATG

glyco Gag  Gag

Virus

glycoMA:  -  -  +  -
Nef:  +  -  -  -
Gag:  +  +  +  -

Infectivity (mU β-Gal/60 ng p24)

- glycoGag  + glycoGag
HIV-1 infectivity enhancement by Nef and glycoGag

**Dependence on producer cell type:**

- Virus producer cell: MOLT4cl8, Raji-CD4
  - Infection levels (Vector, glycoGag)

**Dependence on clathrin-mediated endocytosis:**

- Non-silencing shRNA
  - sh_AP2M1-i
- Anti-actin
- Anti-AP2M1

**Infectivity Levels:**

- Nef+ and Nef- glycoGag
  - Cells: 1, 2
  - Actin and Clathrin expression
  - Infectivity comparison with Nef shRNA

**Summary:**

- HIV-1 infectivity is enhanced by Nef and glycoGag.
- Dependence on producer cell type.
- Dependence on clathrin-mediated endocytosis with shRNA and antibody analysis.
- HIV Nef and MLV glycoGag similarly enhance HIV-1 infectivity
- Effects are similarly dependent on producer cell type
- Effects exhibit a similar reliance on clathrin-mediated endocytosis

<table>
<thead>
<tr>
<th>Protein Symbol</th>
<th>Gradient Fraction</th>
<th>Total Spectrum Count</th>
<th>% Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERINC3</td>
<td>8</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>STOM</td>
<td>8</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>PFKP</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>SERINC3</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
Inhibition of SERINC incorporation by Nef correlates with infectivity enhancement

**Enhancement of HIV-1 infectivity**

Inhibition of SERINC incorporation by Nef correlates with infectivity enhancement.
Nef and glycoGag remove SERINC5 from the cell surface

Effect on subcellular localization

Effect on cell surface expression

SERINC5-mCherry + empty vector

SERINC5-mCherry + glycoGag

SERINC5(iHA) + vector

SERINC5(iHA) + NefSF2

SERINC5(iHA) + glycoGag
Exogenous SERINC5 specifically blocks HIV-1 Env-mediated infectivity

Virus morphogenesis

Virus infectivity

Env: HIV1<sub>HXB2</sub>

Env: VSV G

100 ng each

500 ng each
Exogenous SERINC5 is counteracted by Nef and glycoGag.
Expression of SERINC\textsubscript{s} in Jurkat cells

![Graph showing expression levels of SERINC\textsubscript{s} in Jurkat cells. The x-axis represents different SERINC\textsubscript{s} (SERINC1, SERINC2, SERINC3, SERINC4, SERINC5, TSG101, HPRT1). The y-axis represents RPKM (Reads Per Kilobase of Transcript Per Million Reads). The graph compares uninfected, Nef+, and Nef- conditions. The peak expression is observed for SERINC5, with Nef+ and Nef- conditions showing varying expression levels.]
Endogenous SERINC3 and SERINC5 synergistically restrict Nef− HIV-1 infectivity

Virus infectivity

**Jurkat**-derived virus

**Macrophage**-derived virus
SERINC depletion has negligible effects on HIV infectivity in the presence of Nef or glycoGag

**Infectivity of JurkatTAg-derived virus**

- **siRNA:**
  - Ctrl
  - SERINC3 + SERINC5

The graph shows the relative infectivity of JurkatTAg-derived virus with different treatments. The y-axis represents relative infectivity, and the x-axis represents conditions: Nef-, Nef+, and gGag.
Exogenous SERINC5 blocks HIV-1 entry
CRISPR/Cas9-mediated knockout of SERINC3 and SERINC5 in Jurkat-TAg cells

**SERINC3**

Gene sequence

Jurkat-TAg S3−/− (1)

| Target site A | TGTGTATCGGATCAGCTTTGCCATGGCCATCT | (1 bp insertion) | TGTGTATCGGCTCT | (20 bp deletion) |

**SERINC 5**

Gene sequence

Jurkat-TAg S3+5−/− (1)

<table>
<thead>
<tr>
<th>Target site A</th>
<th>GGTGACACCTGTGAGAAGCTGGTGATATT</th>
<th>.../11.5 kb/...</th>
<th>GACAGGCCACACTCGGCGCTTTTACAATTACAGGGCT</th>
<th>Target site B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GGTGACACCTGTGAGAAGCTGGTGATATT</td>
<td>.../11.5 kb/...</td>
<td>AATATCCCACGGGCGCTTTTACAATTACAGGGCT</td>
<td></td>
</tr>
</tbody>
</table>
Infectivity of Nef⁻ HIV-1 produced in SERINC knockout JurkatTAg cells

HIV-1-GFP (Nef-)

produced in parental JurkatTAg cells

produced in SERINC3/5 -/- double-KO cells

JurkatTAg-derived virus producer cells
Nef and glycoGag have negligible effects on HIV infectivity in double-KO cells lacking SERINC3 and SERINC5.
Re-expressed SERINC3s restrict HIV-1 infectivity

![Graph showing relative infectivity of Nef virus with different conditions: plus vector, plus SERINC3, plus SERINC5, plus SERINC3 SERINC5. The graph indicates that reconstituted double-KO virus producer cells express SERINC3 and SERINC5, which restrict HIV-1 infectivity.]
Nef counteracts inhibition of HIV-1 replication by SERINC3 and SERINC5

Infected JurkatTAg cells

Infected CD4^{high} JurkatTAg cells

parental JurkatTAg cells

double-KO cells

double KO- cells/ reconstituted

Nef+  Nef-
+ Nef or MLV glycoGag

- Nef

Producer cell

Target cell