HIV-1 and Sickle Cell Disease

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NIAID, NIH
Sickle Cell Disease

• Affects about 100,000 people in US
• Occurs in about 1 of 500 African American births (~1000 per yr)
• Occurs in 1 of 1,000 to 1,400 Hispanic American births.
HbS Polymer

(Tavassoli, M)
Patophysiology

- Sickle erythrocyte membrane damage
- Adhesion of sickle erythrocytes to endothelium
- Hemolytic vasculopathy
- Chronic inflammatory response
- Chronic hypoxia
- Splenic atrophy
• 2/73 SCD pts (2.7%) exposed to >1300 units unscreened blood 1978-84 became HIV +
• 8/101 pts (7.9%) Tx’d 1978-88 became HTLV-1 +
• P = 0.15 Pearson chi square; 0.20 Fisher exact
• “Our analysis suggests a low risk for HIV infection in SCD pts transfused before HIV donor screening”
• 5 university centers
• 8/18 HIV+ Hb SS pts (44.4%) long-term non-progressors (none on HAART)
• 5/36 HIV+ control pts (13.9%) long-term non-progressors
• P = 0.013 Pearson chi square
## Reduced Cholesterol Levels in African-American Adults with Sickle Cell Disease

Jalmie Shores, MD; John Peterson, PhD; Dorothy VanderJagt, PhD; and Robert H. Glew, PhD
Albuquerque, New Mexico and Galveston, Texas

### Table 1. Concentrations of Lipids in the Serum of Men and Women with Sickle Cell Disease (SCD) and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCD</td>
<td>n</td>
<td>Controls</td>
<td>n</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 (11)*</td>
<td>16</td>
<td>43 (8)</td>
<td>736</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>147 (42)</td>
<td>16</td>
<td>200 (75)</td>
<td>736</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>42 (16)</td>
<td>9</td>
<td>45 (16)</td>
<td>453</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>68 (28)</td>
<td>9</td>
<td>121 (58)</td>
<td>443</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>102 (34)</td>
<td>12</td>
<td>194 (215)</td>
<td>645</td>
</tr>
</tbody>
</table>

* Mean (SD); NS, Not significant (p>0.05).
Mechanisms?

- Hypoxic response
- Inflammatory response
- Hypocholesterolemia
- Others?
HIV-1 Life Cycle

DENDRITIC CELL

ENTRY

CYTOPLASM

REVERSE TRANSCRIPTION

NUCLEUS

INTEGRATION

TRANSCRIPTION

TRANSLATION

BUDDING

ASSEMBLY

MATURATION

VIRAL PROTEINS

APOBEC3G

UNCOATING

REVERSING TRANSCRIPTION

TRIM5α

CyPA

VIF

Cul5-E3

Vpu, Cul5-E3

Gag, env, LIP5

MATURATION

BUDDING

Budding

ASSEMBLY

CyPA

Rev,
DHS, hRIP, SAM68

SPLICING
RNA TRANSPORT
PROCESSING

INTEGRATION

CELL FACTORS

TRIM5α

APOBEC3G

Vpu

Tetherin

Int, Vpu, PARP1

Tat, LTR, CDK9, cycT1, Sp5, CDK2

CyPA

Pol, DRB1

Nekhai and Jerebtsova,
*Curr.Opin.Mol. Therapy*, 2006
Model of Regulation of HIV-1 Transcription by PP1

- HEXIM1
- Cyclin T1
- CDK9
- S175P
- T186-P
- 7SK RNA
- PP1
- Tat
- RPII
- CTD
- CDK9
- T186-P
- NUCLEUS
- CYTOPLASM
Replication of HIV-1 Luciferase Virus is Reduced in PBMCs Isolated from Sickle Cell Patients and Cultured at 3% Oxygen.
Hypothesis for SCD Protection from HIV-1

• SCD causes recurrent ischemia/hypoxia which may affect HIV-1

• SCD causes decreased expression of hepcidin which may slower rates of HIV-1 replication

• Lower cholesterol levels at SCD may affect HIV-1 entry (lipid rafts) and transcription (SREBP-2/THII-I – Dr. Hildreth)

• Activation of heme oxygenase inhibits HIV-1 in macrophages
Physiological concentration of O₂ is 3-6% in the peripheral tissues (Meyron-Holtz et al., Science 2004)
HIV-1 is Suppressed at 3% O₂

A

HIV-1 Luc

Luciferase activity, % of control

CEM
THP-1
PBMC

B

U1 Monocytes

p24, pg/ml

 CEU1, 21% O₂, -TNF
 CEU1, 3% O₂, -TNF
 CEU1, 21% O₂ + TNF
 CEU1, 3% O₂ + TNF

Charles et al, J Cell Physiol. 2009
CDK9 Activity is Modulated at 3% O<sub>2</sub>

**A**

O<sub>2</sub>, % 21 3

**WB**

CDK9

**WB**

tubulin

O<sub>2</sub>, % 21 3

**WB**

cyclin T1

**WB**

α-tubulin

**B**

IP: cyclin T1 IgG

O<sub>2</sub>, % 21 3

(³²P)

→ CTDo

→ CTDa

**C**

IP: IgG CDK9

O<sub>2</sub>, % 21 21 3

**WB**

→ IgG

→ CDK9

**D**

- 21% O<sub>2</sub> IC<sub>50</sub>=0.17 μM
- 3% O<sub>2</sub> IC<sub>50</sub>=3 μM

HIV-1 Transcription, % of untreated control

Charles et al, J Cell Physiol. 2009
Hypoxia and Protein Phosphatase-1

PP1 is repressed during hypoxia (Taylor et al., PNAS 2000) through increased interaction with NIPP1 (Comerford et al, J Cell Physiol. 2006)
Hypoxia Deregulates Protein Phosphatase-1

A. IP: NIPP1
   WB: NIPP1

B. Phosphatase Activity, Arbitrary Units
   - α-NIPP1
   - IgG

C. Phosphatase Activity, % of AtmosO2 control
   - 21% O2
   - 3% O2

Time Intervals of Trypsin Treatment
PP1 Activity Is Lower in Cells Cultured at 3% Oxygen
PP1 Targets CDK9 Ser 175

A

B

C

D

E

Ammosova et al., PLOS One 2011
Iron Uptake and Recycling
Ferroportin Q248H

- *Fpn* encodes a multiple transmembrane domain protein, involved in iron export
- Pathogenic mutations: associated with iron loading
- Q248H (c.DNA 744G →T)
- Q248H unique to Africans and is associated with a tendency to iron loading
Effect of Ferroportin and Hepcidin on HIV-1

A

Ferroportin (FPN) and Hepcidin

B

Transcription Activation (Fold)

CD4

FPN

HIV-1 LTR

HIV-1 LTR + Tat

CD4

FPN WT

C

FAC

Hepeid

Primary Monocytes

CD4

FPN

α-tubulin

RT activity (counts)

CD4+ T cells

FAC

Hepeid

Xu et al., Retrovirology 2010
### Sensitivity of Ferroportin Q248H Mutant to Hepcidin

#### A

<table>
<thead>
<tr>
<th>Hepcidin (μM)</th>
<th>Fpn Q248H</th>
<th>Fpn WT</th>
<th>Fpn C326Y</th>
<th>GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>0.01</td>
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<tr>
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<td></td>
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</tbody>
</table>

 WB: anti-α-tubulin

WB: anti-myc

Fpn

Tubulin

1 2 3 4 5 6 7 8 9 10 11 12 13

#### B

**Ferroportin-EGFP**

- **FPN WT**
- **Q248H**
- **C326Y**

% of untreated control vs. Hepcidin, μM

0 20 40 60 80 100 120

0 0.05 0.1 0.15
**Ferroportin Q248H Mutant restricts HIV-1 Replication**

**A**

![Graph](image)

**B**

<table>
<thead>
<tr>
<th>HIV-1 Luc</th>
<th>Iron+AA</th>
<th>Hepcidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
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</table>

![Bar graph](image)
Conclusions/Directions

• HIV-1 replication is slower in SCD-derived PBMCs; in cell cultured at 3% O_2 or cells expressing ferroportin

• Activities of host cell CDK9 and PP1 are modulated at 3% O_2

• Hepcidin induces HIV-1, and Fpn 248, insensitive to hepcidin, protects against HIV-1

• In future, analyze PP1 activity and hepcidin expression an in SCD
• Use iPSC technology to study Fpn Q248
• Analyze effect of cholersterol to HIV-1 in SCD
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