Envelope Variation as a Primary Determinant of Lentiviral Vaccine Efficacy

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The EIAV Virus

- **Envelope:** SU, gp90, TM, gp45
- **Integrase-IN:** p30
- **Reverse Transcriptase-RT:** p66
- **Protease-PR:** p12
- **dUTPase-DU:** p15
- **ΔS2 EIAV_{D9}**

**Viral RNA**

**Lipid Bilayer**

**Nucleocapsid-NC** p11

**Matrix-MA** p15

**Core-CA** p26

**Pol**

**Gag**

**Env**
Typical Clinical Course of EIAV Infections

Disease episode: Rectal Temperature 39°C & Platelet/ml < 100,000
Similarities Between EIAV & HIV

- Transmitted via blood
- Macrophage/monocyte tropism
- Diverse Env quasispecies & antigenic heterogeneity
- Envelope architectural characteristics
  - Extensive glycosylation
  - Immune decoys

Similarities ⇒ significant to initial virus exposure:
  - Key factors most relevant to vaccine efficacy
## Efficacy of Attenuated EIAV Vaccine Trials

<table>
<thead>
<tr>
<th>EIAV Vaccine strain/dose</th>
<th>Route</th>
<th>Plasma viral RNA levels (DOC)</th>
<th>Interval to Challenge (months)/dose</th>
<th>Protection from clinical disease (%)</th>
<th>Protection from detectable infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIAV&lt;sub&gt;D9&lt;/sub&gt;/10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>IM</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6/LDME</td>
<td>8/8 (100%)</td>
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<td>IM</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6/LDME</td>
<td>12/12 (100%)</td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td>EIAV&lt;sub&gt;D9&lt;/sub&gt;/10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>IV</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6/3000 HID</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)*</td>
</tr>
<tr>
<td>Control</td>
<td>NA</td>
<td>NA</td>
<td>LDME/3000 HID</td>
<td>0/16 (0%)</td>
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</tr>
</tbody>
</table>

*Plasma & Tissues
LDME, Low Dose Multiple Exposure
HID, Horse Infectious Doses
Working hypothesis:

Envelope is a primary determinant of viral pathogenesis & vaccine efficacy

Challenge Strains: Variant Envs in Identical Proviral Backbones
Development of Challenge Strains with Defined, Increasing Levels of Variation

Env (gp90): $\text{EIAV}_{\text{PV}} = \text{EIAV}_{\text{D9}}$

Env (gp90): $\text{EIAV}_{\text{UK3}} = \text{EIAV}_{\text{D9}}$

Divergence (%) from EIAVPV

I  II  III  IV  V  VI  Inapparent
30  50  230  260  645  740  1219  (DPI)
**In Vitro Analyses: Replication Kinetics**

**Fetal Equine Kidney cell infections:**

**In Vivo Analyses: Clinical Disease**

(4 Ponies/EV strain)

100%, Peak viral loads \( \sim 10^5 \text{ to } 10^7 \) cp RNA/ml

**Replication Dynamics**

Steady state viral loads \( \sim 10^4 \) cp RNA/ml

**Immunogenicity/Serology (6MPI)**

- Env-Spec Titer \( \sim 10^5 \)
- Avidity \( \sim 43\% \)
- Conformation Ratio \( \sim 1.1 \)
Challenge Strains were Neutralization Distinct

Experimentally-Infected Pony Sera

Virus Strain
- EV0
- EV6
- EV13

10^1 10^2 10^3

Challenge Strains were Neutralization Distinct
**Immunization & Challenge**

**Inoculation (IM) EIAV_{D9}:**
24 EIAV naïve ponies

10^3 TCID_{50} 10^3 TCID_{50}

**Challenge (IV):**
3 groups of 8 vaccinated 3 groups of 6 EIAV naïve (control) ponies

10^3 TCID_{50} EVO, EV6 or EV13

**Monitor**
- Clinical signs
- EIAV-specific antibody response
- EIAV-specific cellular response
- Plasma viral load

**Monitor**
- Clinical signs
- EIAV-specific antibody response
- EIAV-specific cellular response
- Plasma viral load
- RT PCR for WT Challenge
Protection from Clinical Disease

Days Post-Challenge

EV0
EV6
EV13

7/8
5/8
3/8

Protection from Clinical Disease
Inverse Correlation of Disease & Divergence

When \( Y \) (Protection) = 0, \( X \) (Divergence) = 23

\[ R^2 = 0.998 \]
\[ P = 0.02 \]
Conclusions

- Envelope variation alone dramatically affected protection from disease.
- Envelope gp90 variation had a significant inverse linear association with protection from disease.
- Envelope gp90 (SU) variation in the absence of gp45 (TM) variation is a critical determinant for vaccine efficacy.
- Ancestral envelope did not confer broad levels of protection.  
  - 6% variation: protection dropped by 25%.
  - 13% variation: protection dropped by 50%. 
Implications

- Minimal envelope variation can pose a major obstacle to lentiviral vaccine efficacy

- Ancestral envelopes as individual immunogens in our system will not confer protection against lentiviral quasispecies

- Multivariant immunogens?
- Consensus immunogens?
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