Use of Feline Herpesvirus-1 (FHV-1) as a Cat Contraceptive Vaccine Vector

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Hypothesis

Feline Herpesvirus-1 (FHV-1) \approx\text{ Mouse Cytomegalovirus (MCMV)}

Canine Herpesvirus (CHV) \approx\text{ Mouse Cytomegalovirus (MCMV)}
Why use Feline Herpesvirus-1 (FHV-1)?

**Safe**
- Species specific. Limited morbidity.
- Readily attenuated.
- FDA-approved vaccine strain.

**Information through homology**
- Herpesvirus family, alpha subfamily, varicellovirus genus.
- (PRV, BHV, EHV > VZV)
- Genes are well-conserved among varicelloviruses.

**Immunogenic**
- Strong Ab response.
- Life-long infection (latency with periodic reactivation)

**Superinfection**
- Immunity does not prevent re-infection.

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**Overview of Strategy**

*Simplified genome of FHV-1 (not drawn to scale)*

Wild Type FHV-1

Attenuated FHV-1

Control

Vaccine
Overview

1. FHV-1 immunogenicity.
   - ELISA development.
   - Strength and longevity of FHV-1 immunity.
2. Identification of immunodominant FHV-1 glycoproteins.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
4. Preparation for breeding study with attenuated, recombinant FHV-1.

High Titer Antibody responses to FHV-1 Vaccine Strain (F-2) and/or Endemic Exposure

Cat cell line (CRKF) infected with wt FHV-1.
Cells lysed with 1% NP-40 detergent.
Lysates coated onto ELISA plates.

High Titer Antibody responses to FHV-1 Vaccine Strain (F-2) and/or Endemic Exposure

Mock lysates

FHV-1 lysates

Slope = 0.07
\( R^2 = 0.12 \)
\( p = 0.01 \)

Slope = -0.003
\( R^2 = 0.00001 \)
\( p = 0.98 \)

Antibody Titers to FHV-1 Increase with Age and are Stable for Several Years After Vaccination

Age at blood draw (years)
Years since most recent vaccination

\( \log_{10} \) normalized antibody titer (% of reference)
Summary of Part 1:
Immunogenicity of FHV-1

- Cats have a wide range of antibody titers to FHV-1 lysate.
- (not shown) Both genders have similar responses.
- The antibody titer increases slowly with age and does not depend on recent vaccination.
- (not shown) Booster vaccination is not required for strong antibody response.

Overview

1. FHV-1 immunogenicity.
2. Identification of immunodominant FHV-1 glycoproteins.
   - Use as carrier protein(s) for GnRH peptide.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
4. Preparation for breeding study with attenuated, recombinant FHV-1.
**Rationale and Assumptions**

1. GnRH carrier should be an FHV-1 protein(s).
2. Any FHV-1 protein is a potential carrier for GnRH.
3. However, some FHV-1 proteins are much more immunogenic than others.
   - Immunodominance.
4. Therefore, if we can identify immunodominant FHV-1 proteins then we likely will have identified the best GnRH carriers.
   - Ample CD4 T cell help

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**Purifying and Testing FHV-1 Glycoproteins for Immunodominant Antibody Responses**

<table>
<thead>
<tr>
<th>SP Epitope Tag</th>
<th>Ectodomain of gB, gC, gD, etc.</th>
<th>Homologous site</th>
<th>Epitope tag (6X His)</th>
<th>gB</th>
<th>gC</th>
<th>gD</th>
<th>gG</th>
<th>gI+gE</th>
<th>gI</th>
<th>gL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone ectodomain (with epitope tags)</td>
<td>Recombinant baculovirus</td>
<td>Infect insect cells</td>
<td>Express protein</td>
<td>Purify protein</td>
<td>Probe for cat antibodies by ELISA</td>
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</tbody>
</table>

**Homomeric**

<table>
<thead>
<tr>
<th>Homomeric</th>
<th>Heterodimers</th>
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<tbody>
<tr>
<td>gB gC gD Elicit Consistent High Titer Ab</td>
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</tbody>
</table>

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**gB, gC & gD Elicit Consistent High Titer Ab**

- Speciﬁc Pathogen Free
- Raven
- Orbitz
- Ballou

**gB, gC & gD Elicit Consistent High Titer Ab**

- Homomeric
- Heterodimers
Summary Part 2: gC, gB and gD are the Best Candidates for GnRH Carrier Proteins

Overview

1. FHV-1 immunogenicity.
2. Identification of immunodominant FHV-1 glycoproteins.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
   - Clinical disease.
   - Clinical chemistry and hematology.
   - Virus shedding.
   - Immunogenicity (antibody response).
4. Preparation for breeding study with attenuated, recombinant FHV-1.

Overview of Strategy

Overview of BAC Mutagenesis (not to scale)

FHV-1 BAC gG

Illumina Sequencing & Confirmation

Transfect DNA into cat cell line

Enzymatic removal of BAC cassette (Cre-Lox)

Confirmation of replication in vitro
A Pilot Study with Attenuated FHV-1 Demonstrates Safety and Immunogenicity

Vaccination:
TK-gI-gE FHV-1

Safety:
No clinical signs of FHV-1 disease.
No changes in clinical chemistry or hematology.
No detectable virus shedding after vaccination or immunosuppression.

Antibody response:

Cats Vaccinated with Attenuated FHV-1 Develop Antibodies to gC, gD and gB

Summary of Part 2: Pilot Study of Safety & Immunogenicity

- FHV-1 BAC sequenced. Very similar to another wt strain.
- FHV-1 TK-gI-gE BAC created and genome is intact (not shown).
- FHV-1 transfection into CRFK cells resulted in infectious virus.
- FHV-1 lost BAC cassette when transfected into Cre+ cells.
- Attenuated FHV-1 causes no clinical signs at 10^6, 10^7 or 10^8 pfu.
- No changes in clinical chemistry or hematology.
- No detectable FHV-1 shedding.
- Antibodies to FHV-1 were elicited with 10^8 pfu > 10^7 > 10^6
Overview

1. FHV-1 immunogenicity.
2. Identification of immunodominant FHV-1 glycoproteins.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
4. Preparation for breeding study with attenuated, recombinant FHV-1.
   - Developing assays to measure antibodies against hormones and hormone receptors (cat anti-GnRH antibodies, anti-GnRH-R, etc.)
   - Engineering redundancy into the vaccine design.

Development of Attenuated FHV-1 Vaccine Strains in Progress

Overall Summary

Part 1 – FHV-1 Immunogenicity
- Established a sensitive FHV-1 ELISA.
- Antibodies appear stable with age and do not require booster vaccination.

Part 2 – Immunodominant FHV-1 Glycoproteins
- Purified 7 glycoprotein monomers or heterodimers.
- Major responses to gC, gB and gD.

Part 3 – Safety and Immunogenicity Study
- Created attenuated TK−gI−gE−FHV-1.
- In pilot study, no clinical signs of FHV-1 disease or virus shedding detected.
- At high FHV-1 doses, cats made antibodies to FHV-1 lysate, gC and gD.

Part 4 – Breeding
- Study preparation in progress.

Thank You!

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Thank You!

- Found Animals
- Population
- 5th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control

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- Subjects

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