


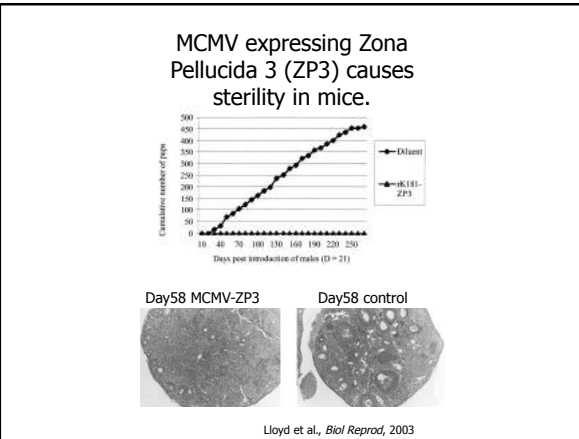


Use of Feline Herpesvirus-1 (FHV-1) as a Cat Contraceptive Vaccine Vector

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 National Jewish Health
 Denver, Colorado

Australian Mouse Plagues

Hypothesis

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

 Canine Herpesvirus (CHV) \approx 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.

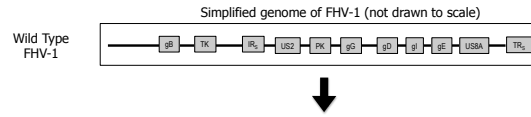
Immunogenic

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

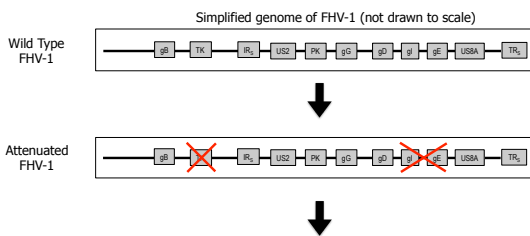
Superinfection

- Immunity does not prevent re-infection.

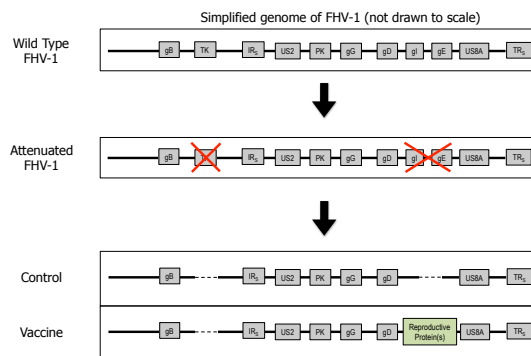
Overview of Strategy



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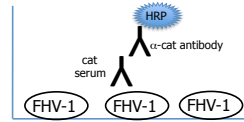


Overview

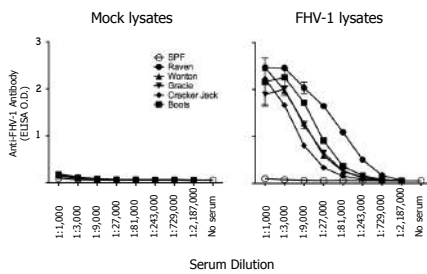
1. FHV-1 immunogenicity.
 - ELISA development.
 - Strength and longevity of FHV-1 immunity.
2. Identification of immunodominant FHV-1 glycoproteins.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
4. Preparation for breeding study with attenuated, recombinant FHV-1.

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

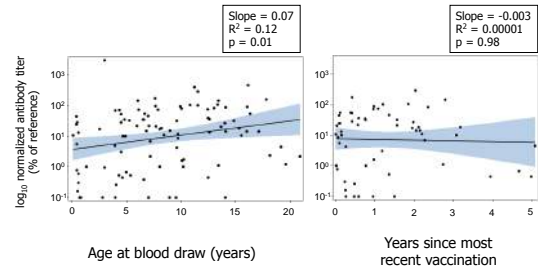
Cat cell line (CRKF) infected with wt FHV-1.
 Cells lysed with 1% NP-40 detergent.
 Lysates coated onto ELISA plates.



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



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



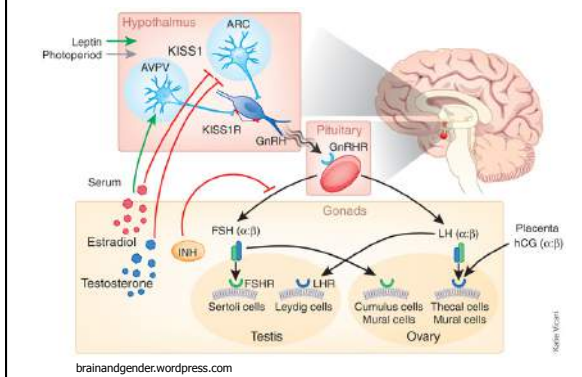
Summary of Part 1: Immunogenicity of FHV-1

- 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

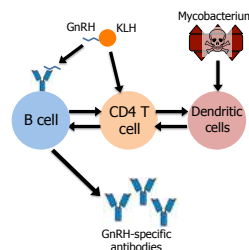
1. FHV-1 immunogenicity.
2. Identification of immunodominant FHV-1 glycoproteins.
 - Use as carrier protein(s) for GnRH peptide.
3. Pilot study with attenuated FHV-1 vector (safety & immunogenicity).
4. Preparation for breeding study with attenuated, recombinant FHV-1.

Potential Reproductive Targets

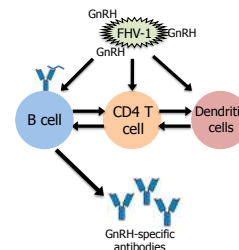


GonaCon™

- Developed at USDA National Wildlife Research Center.
- GnRH peptide + carrier protein (KLH) + adjuvant (mycobacterium in oil).
- Approved by the EPA for contraception of white-tailed deer.



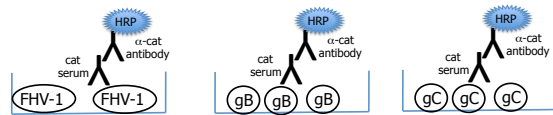
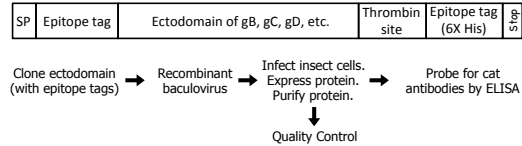
FHV-1 Contraceptive Vaccine



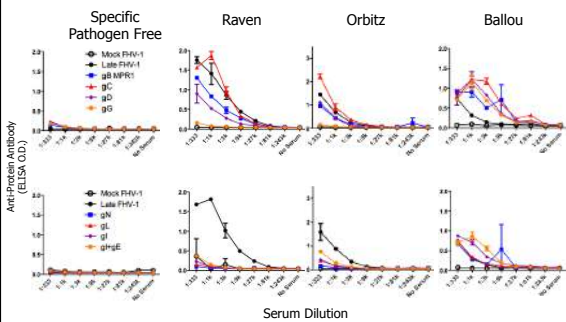
Rationale and Assumptions

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

Purifying and Testing FHV-1 Glycoproteins for Immunodominant Antibody Responses



gB, gC & gD Elicit Consistent High Titer Ab



gB, gC & gD Elicit Consistent High Titer Ab

Cat	Mock FHV-1	Homomeric					Heterodimers		
		gB	gC	gD	gG	gN	gI+gE	gI	gL
Raven	-	++	+++	+	+/-	-	+/-	+/-	+/-
Einstein	-	++	+++	+/-	+/-	-	-	-	-
Trevor	-	++	+++	-	-	-	++	+	-
Boo	-	++	+++	++	+/-	+/-	+/-	+/-	+/-
Duke	-	++	+++	++	-	-	++	-	-
Orbitz	-	++	+++	+	-	-	++	+	+
Kali	-	+++	+++	+++	-	-	+	+/-	-
Lukie	-	++	+++	+++	-	+	++	++	++
Jonas	-	++	+++	+++	-	+/-	++	+	+
Socrates	-	++	+	+++	++	+/-	+	+/-	+/-
Bandit	-	++	+++	+++	+	+/-	++	+	+
Chica	-	++	+	+	++	+/-	+/-	+/-	+/-
Tinker	-	++	+/-	++	+	+/-	+	+/-	+/-
R.C	-	++	+/-	+	+/-	+	+	+	+/-

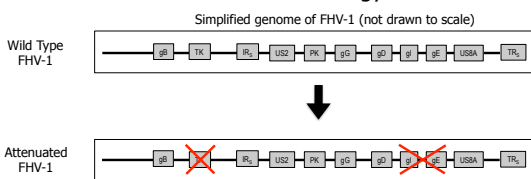
Summary Part 2: gC, gB and gD are the Best Candidates for GnRH Carrier Proteins



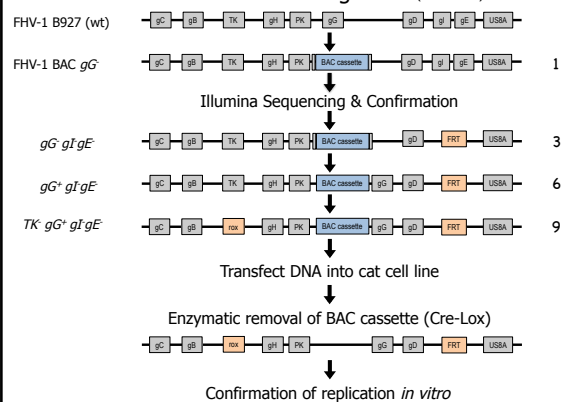
Overview

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)



A Pilot Study with Attenuated FHV-1 Demonstrates Safety and Immunogenicity

Vaccination:
TKglgE FHV-1

Safety:

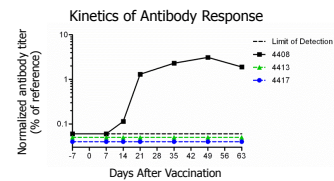
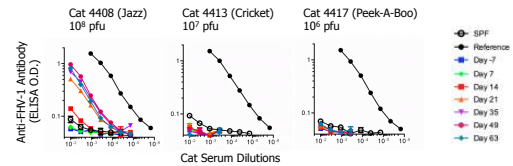
No clinical signs of FHV-1 disease.

No changes in clinical chemistry or hematology.

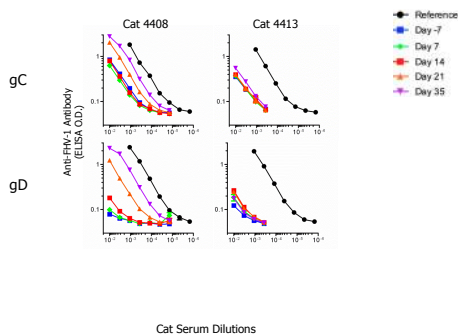
No detectable virus shedding after vaccination or immunosuppression.

Antibody response:

Kinetics of Antibody Response to FHV-1



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



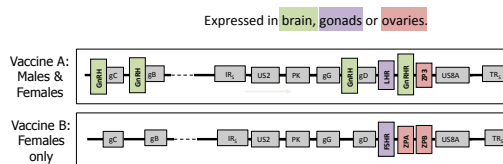
Summary of Part 2: Pilot Study of Safety & Immunogenicity

- FHV-1 BAC sequenced. Very similar to another wt strain.
- FHV-1 *TKglgE* 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!

Pippa Marrack & John Kappler



Alana Montoya



Jennifer Matsuda & Bicheng Zhang



Found Animals Foundation D0809-S17	Thank You!	NJH – Biostatistics Anna Forssen Matt Strand
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Garrick Talmage Cameron Pywell Lucy Smith Janet Mostrom	NJH Proteomics Core Nichole Reisdorph Roger Powell	Hagman Lab Jim Hagman Julita Ramirez
Tomasz Sosinowski Eric Clambey Kelly Bakke Andrea Edwards	NJH BRC & IACUC Sai Tummala Ross Kedl	DNA sequencing Jill Castoe Nathan Kummer
	Pre-Clinical Research Services, Inc. Don Maul Maralee McVean	SNL Weekend Update Seth Meyers Amy Poehler
	Jasper Animal Hospital Donald Dodge Teresa Campbell	Subjects

Oregon Humane Society

Client Information Name: Phobia Date: 1/22/08 Client Code: SPC455888A Phone: (503) 255-1111 Address: 11111 SW 11th Ave City: Beaverton, OR 97005 Email: phobia@oahs.org	Animal Information Name: Phobia Breed: Pit Bull Age: 2 years 1 mo Sex: Female Color: Black Microchip: 985123456789012345 Spayed/Neutered: Yes	Medical Information Vaccinations: Rabies, Distemper, Parvovirus, Bordetella, FVRCP, Leptospirosis, FeLV, FIV, Heartworm Microchip: 985123456789012345 Spayed/Neutered: Yes
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Oregon Humane Society Case # OHS-08-01234	Kennel Card Phobia Date received: 1/22/08 Breed: Pit Bull Color: Black Sex: Female Age: 2 years 1 mo Microchip: 985123456789012345 Spayed/Neutered: Yes	OHS
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Raven

