DEVELOPMENT OF ATTENUATED FELINE HERPESVIRUS-1 AS A CAT CONTRACEPTIVE VACCINE VECTOR

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Several cat contraceptive vaccines have previously been developed and tested, but their efficacy rate and duration of effectiveness are too low for practical use. Feline herpesvirus-1 (FHV-1) is a close relative of varicella zoster virus (VZV) and establishes lifelong latent infection of cats. We hypothesize that attenuated FHV-1 that expresses recombinant reproductive proteins and peptides will induce strong and long-lasting immune responses that cause long term or permanent infertility in cats. Using serum from cats that have been naturally exposed to FHV-1 and/or vaccinated with live attenuated FHV-1, we established a sensitive ELISA for detection of anti-FHV-1 antibodies. In a cross-sectional study of 100 client-owned cats, we examined the effects of age and vaccine history and found that repeated vaccination and recent vaccination are not required for maintenance of FHV-1 antibody titers. To induce antibodies to gonadotropin-releasing hormone (GnRH), a 10 amino acid peptide, we will insert the GnRH sequence into the FHV-1 genome so that GnRH is expressed as a fusion protein with the most immunogenic FHV-1 proteins. We expressed and purified 8 FHV-1 glycoproteins in an insect cell expression system, then tested cat serum for antibodies to each of these by ELISA. We identified glycoproteins gC, gB and gD as the most immunogenic FHV-1 proteins, in cats with either high or low overall antibody titers to FHV-1.

Using bacterial artificial chromosome (BAC) mutagenesis, we attenuated FHV-1 by deleting three known virulence genes, thymidine kinase *(TK)* and glycoproteins *gE* and *gI*. We then reconstituted infectious FHV-1 virus by transfecting the FHV-1 BAC into a cat cell line. In a pilot safety and immunogenicity study, three cats were vaccinated with the attenuated FHV-1. No clinical signs of disease were observed in any of the cats and no virus shedding was detected, even after immunosuppression, demonstrating a significant degree of virus attenuation. However, a dose-dependent antibody response was still observed. We are currently engineering two FHV-1 vaccine vectors to express multiple reproductive proteins, including GnRH, GnRH receptor and zona pellucida 3 (ZP3), and developing T cell and antibody assays to detect immune responses to these reproductive proteins. The attenuated, recombinant FHV-1 vectors will then be tested for the ability to cause and sustain infertility in female cats.

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