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Short communication

Infectious feline herpesvirus detected in distant bone and tendon following mucosal inoculation of specific pathogen-free cats

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ABSTRACT

Emerging evidence suggests that cats infected with feline herpesvirus-1 (FHV-1) may experience a brief viremic phase. The objective of this study was to determine whether natural routes of FHV-1 inoculation could result in viremic transmission of infectious virus to connective tissues (cortical bone, tendon). Three specific pathogen-free cats were experimentally inoculated with FHV-1 *via* a combined mucosal (oronasal, ocular) route. Cats were euthanized at the peak of clinical signs to aseptically harvest tissues (cortical bone, tendon, trachea/tongue) for co-culture with a susceptible cell line to promote spread of infectious virus. Viral infection of Crandall-Rees feline kidney cells was microscopically visualized by cytopathic effect (CPE). Additionally, co-culture DNA was extracted either at the point of CPE or 16 days of culture without evidence of CPE, to amplify FHV-1 glycoprotein B gene using real-time PCR. Infectious virus was detected in distant cortical bone (two cats, moderate to severe clinical signs) and tendon (one cat, severe clinical signs). Direct infection of mucosal (trachea, tongue) tissues also was confirmed in these two cats. In contrast, all co-cultured tissues from the third cat (mild clinical signs) were negative for FHV-1 by CPE and PCR. Results of this study demonstrated that early primary FHV-1 viremia may be distributed to distant connective tissues.

1. Introduction

Feline herpesvirus-1 (FHV-1) causes viral rhinotracheitis in cats (Gaskell and Povey, 1977; Hoover et al., 1970; Milek et al., 1976). Bovine herpesvirus-1 (BHV-1), equine herpesvirus-1 (EHV-1), human herpes simplex, and varicella-zoster viruses also have an affinity for mucous membranes, frequently resulting in respiratory and reproductive diseases, as well as lifelong infection *via* latency with possible reactivation (Diamond et al., 1999; Gilden et al., 2011; Gibbs and Rweyemamu, 1977; Wilks and Studdert, 1976; Tronstein et al., 2011; Tovaru et al., 2009). FHV-1 replication occurs within respiratory, oral or ocular epithelium causing cell rupture, necrosis, ulceration, and inflammation (Hoover et al., 1970; Milek et al., 1976; Sandmeyer et al., 2005). Most cats become latently infected (typically within neurons, especially the trigeminal ganglia), lifelong carriers subject to intermittent stress-induced reactivation with virus shedding (Weigler et al., 1997; Gaskell and Povey, 1977; Reubel et al., 1993; Tham and Studdert, 1987).

Herpesvirus viremia has been reported in several different veterinary species (Fuchs et al., 1999; Wilsterman et al., 2011; Nauwynck and Pensaert, 1995) and humans (Diamond et al., 1999; Kimura et al., 1991). It is possible that FHV-1 infection may spread beyond primary infected tissues (ocular, respiratory) because infectious virus was isolated from blood cells of young FHV-infected cats (Tham

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and Studdert, 1987). Additionally, polymerase chain reaction (PCR) amplification of segments of viral DNA (without evidence of infectivity) also was described (Gaskell et al., 2007). A predisposition for local infection of skeletal growth regions (*e.g.* turbinates) was reported following experimental intravenous FHV-1 inoculation of young cats (Hoover and Griesemer, 1971a,b).

Although nasal turbinate bone infection following local mucosal challenge and viremia following intravenous FHV-1 inoculation have been reported, the presence of infectious virus in connective tissues remote from primary mucosal infection sites has not been documented (Hoover et al., 1970; Hoover and Griesemer, 1971a,b; Tham and Studdert, 1987; Gaskell et al., 2007). The purpose of the study reported here was to determine whether infectious virus could be isolated from distant bone and connective tissues following a natural route (mucosal) of FHV-1 inoculation in cats. To accomplish this, (1) three specific pathogen-free (SPF) cats received a combined oronasal, ocular FHV-1 challenge; (2) distant bone and tendon as well as local mucosal (trachea, tongue) tissues were aseptically harvested following euthanasia; (3) individual tissues from each cat were co-cultured with Crandall-Rees feline kidney (CRFK) cells to promote viral spread monitored by cytopathic effect (CPE); (4) DNA was extracted from each co-culture; and (5) a conserved region of FHV-1 genomic DNA was amplified by real-time PCR.

2. Materials and methods

2.1. Generation and collection of infectious tissues

2.1.1. Virus inoculation

The Michigan State University Animal Care and Use Committee approved all animal use protocols. Three 3-month-old female SPF cats (O3, M3, M4) were mucosally inoculated with 1 mL (2×10^5 $TCID_{50}$ per mL) of C-27 wild-type strain FHV-1 infectious stock; 0.165 mL bilaterally in both conjunctival fornices and nares plus 0.33 mL per os (Maes et al., 1984; Rota et al., 1986; Tham and Studdert, 1987).

2.1.2. Clinical scoring

Cats were evaluated daily by one veterinary technician using the Supplemental assay method for scoring feline rhinotracheitis virus in cats following challenge (Ames, Iowa: Center for Veterinary Biologics and National Veterinary Services Laboratories, 2001) to assign a clinical score. According to the animal use protocol, any cat with a clinical score $\geq$10 would be euthanized. Tissues were harvested on day 11 or earlier if necessary, in accordance with results of clinical scoring.

2.1.3. Tissue harvesting and culture preparation

Immediately following euthanasia, small fragments of local mucosal (cranial trachea and tongue) and distant connective tissues [cortical bones (O3 rib, M3 and M4 fibula) and Achilles tendons] were aseptically harvested in a sterile surgery suite from each cat using separate sterile equipment and rinsed three times in sterile saline. Mucosal tissues (trachea, tongue), cortical bone, or tendon from 3 different FHV-infected cats were aseptically minced into approximately 1 mm^3 pieces using separate sterile gloves, bone rongeurs, and surgical blades immediately prior to introduction of 25 mg into separate culture flasks.

2.2. Cell culture

Cells from a susceptible cell line (CRFK) were grown as previously described (Crandell and Despeaux, 1959; Milek et al., 1976; Maes et al., 1984). Forty-eight hours prior to co-culture, fifteen 25 cm^2 flasks were seeded with 400,000 CRFK cells to attain approximately 80% confluency when one of five test conditions were created by sterile addition of (1) media alone (negative control group), (2) minced local mucosal tissues (tracheal and tongue) from FHV-infected cats, (3) fragmented distant cortical bone from FHV-infected cats, (4) minced distant tendon from FHV-infected cats, or (5) media containing 100 μL C-27 FHV-infected cell culture supernatant (positive control group). Three replicates were performed for each of the five test conditions (Crandell and Despeaux, 1959; Milek et al., 1976).

2.2.1. Cytopathic effect (CPE)

Cell co-cultures were evaluated once daily using phase contrast microscopy for evidence of CPE; the number of days from challenge to CPE was recorded for each co-culture flask (Crandell and Despeaux, 1959; Milek et al., 1976).

2.3. PCR amplification

2.3.1. DNA extraction and quantification

The DNA was extracted from CRFK cell co-cultures at the time CPE was noted or 16 days of culture (if CPE was not identified) using a QIAmp DNeasy Blood and Tissue kit (Qiagen Inc.), and quantified spectrophotometrically using a NanoDrop 1000 (Thermo Scientific).

2.3.2. Real-time PCR amplification

A highly conserved, 81-bp glycoprotein B, open reading frame FHV-1 DNA gene sequence was amplified according to a published report. Reactions were performed in triplicate using an automated thermocycler (Applied Biosystems) and mean threshold cycles (C_T) were recorded (Applied Biosystems) (Vogtlin et al., 2002).

3. Results

3.1. Tissue harvest and cytopathic effect

Cats were euthanized on post-inoculation day 11 (M3, M4) or 7 (03); clinical scores and number of days from co-culture initiation to CPE are recorded in Table 1. Tissues from cat O3 were stored at −80 °C then rapidly thawed at 37 °C once tissues from the cats M3 and M4 were available.

3.2. Real-time quantitative PCR amplification

Real-time quantitative PCR results of DNA extracted from tissue CRFK cell co-cultures are shown in Fig. 1.