Replication of a Strain of Feline Calicivirus in Organ Culture

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With 1 Figure

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Summary

The zwitterionic buffer HEPES was used successfully to maintain organ cultures of kitten tongue and trachea for 10–15 days in vitro. A strain of feline calicivirus grew to high titres in both tongue and tracheal explants but it was not possible to ascribe specific histological changes to virus infection in either tongue or tracheal organ cultures.

Cells in tongue explants retained specific virus receptors for at least six days in vitro and virus grew to high levels in the cultures infected six days post-initiation of explants.

These results suggest that at least some strains of feline caliciviruses have tropism for lingual epithelium as well as respiratory tract tissues.

1. Introduction

Tissues which are the site of virus multiplication in the natural host are of particular interest for virological investigation. These tissues are more likely to support growth of viruses which do not multiply in other systems in vitro.

The bulk of the work with organ culture systems has employed ciliated respiratory epithelial explants. The activity of the cilia in such explants allowed a rapid estimate of the functional integrity of the culture and the growth of some viruses could be linked with a loss of ciliary activity. In addition to ciliated epithelium, viruses have been grown in other tissues e.g. embryonic skin, salivary glands, palate, heart muscle and intestine. These tissue segments are more difficult to maintain for long periods in culture and there is no reliable index of their continuing functional state.

Only recently have organ culture techniques been used to study the tissue tropism of viruses of veterinary importance. PAY (14) propagated foot-and-mouth disease virus (FMDV) in organ culture of adult bovine tongue epithelium and
JONES (10) reported growth of FMDV in mouse pancreas. CAMPELL and co-workers (4, 5) were able to grow adenovirus in canine tracheal cultures and bovine parainfluenza 3 virus in calf trachea. More recently, REED et al. (16) have succeeded in growing a bovine rhinovirus in organ cultures and growth of FMDV in non-ciliated epithelium of bovine origin has been reported (20).

In recent years, feline caliciviruses have been associated with a diversity of feline disease syndromes. As well as the classical clinical signs of upper respiratory tract infection caused by these viruses, other evidence has incriminated feline caliciviruses as a cause of ulcerative glossitis. This clinical sign may be present alone or in association with conjunctivitis and/or respiratory tract infection (11). In work reported by LOVE (12) using a strain of feline calicivirus which produced tongue ulceration in natural and experimental infections, evidence was presented which suggested that strains of feline calicivirus may have a selective tropism for tongue epithelium.

This paper reports the establishment and use of organ cultures of feline ciliated respiratory and lingual mucous membrane to study selective tissue tropism of a strain of feline calicivirus which causes ulcerative glossitis as the result of natural and experimental infection.

2. Materials and Methods

2.1. Use of Zwitterionic Buffers

In a previous communication, LOVE (13) found that growth of primary kitten kidney cell monolayers and plaque production with the feline calicivirus could be effected using the zwitterionic buffers TES and HEPES to maintain the pH in 'open' Petri dish culture system. These buffers were employed for the maintenance of pH for organ culture explants. Preliminary experiments suggested that HEPES buffer was marginally better than TES. Explants survived for up to 15 days in HEPES buffer and did not allow the migration of fibroblasts from the base of the explants as was seen in explants grown in the presence of TES buffer.

2.2. Culture Medium

Eagle’s medium (Gibco G-11) supplemented with 0.8 per cent (w/v) bovine serum albumen, 100 µg/ml streptomycin, 100 µg/ml penicillin and 1 µg/ml Fungizone. HEPES buffer was used at a final concentration of 23 µM. 1 M NaOH was used for correction of pH to 7.4.

2.3. Animals

Kittens 8 to 20 weeks old were used as donor animals.

2.4. Virus

The strain of feline calicivirus was twice plaque-purified and a virus stock with a titre of $10^5$/ml plaque forming units (PFU) was used in all experiments.

2.5. Preparation of Petri Dishes

Two ml growth medium was added to each 60 mm Petri dish used for tracheal organ cultures. This allowed cilia to protrude above the surface of the medium. One ml growth medium was necessary to submerge tongue explants. Grids were cut into the surface of the Petri dish with a sharp scalpel blade after fluid had been added to the dish. If fluid was added to the dish after the grids had been made air bubble formation in the grids resulted in unsatisfactory anchorage of explants.