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Feline calicivirus subunit vaccine – a prototype

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Summary

A vaccine was prepared from a subunit component, antigenically similar to the whole feline calicivirus (FCV) particles. Despite the limited number of animals available for this study we were able to demonstrate that the vaccine protected cats when challenged with a virulent strain of the virus while the non-vaccinates kept as controls developed clinical and histopathological symptoms of the calicivirus disease.

feline calicivirus; subunit vaccine

Introduction

Since the original isolation of the virus by Fastier [7] one factor of particular interest about feline caliciviruses has been the antigenic variation which exists between various strains. Preliminary attempts to classify the viruses serologically suggested that numerous distinct serotypes existed [2,3,5]. Subsequent cross-neutralization studies of 46 isolates, however, led Povey [16] to suggest that there were in fact considerable antigenic relationships in the group and that the various isolates might be regarded as members of a single serotype. This study was later extended to in vivo situations for a total of 8 isolates by Povey and Ingersoll [18] who postulated that such relationships might be reflected in cross-protection in cats infected with FCV.

A similar development occurred towards vaccination against virus-induced respiratory diseases in cats. Thus, at first it was considered that the antigenic diversity of FCV isolates, together with the poor immunogenicity of feline herpes virus (FHV), preclud-

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ed the manufacture of an effective vaccine against these viruses. However, when the extent of cross-protection following infection with FCV became apparent, serious attempts were made to produce vaccines.

Several of these vaccines have been produced [1,6,9] and the side effects of both inactivated FHV-FCV vaccines and live modified vaccines have already been discussed [19].

However, there is an increasing conviction that potent vaccines could be made from the non-infectious virus subunit components which are antigenically related to the whole virus. In this way, the risks associated with both killed and live attenuated vaccines can be averted. Some of such subunit vaccine prototypes already developed include human influenza [10], hepatitis B [20] and rabies subunit vaccines [4].

In earlier studies, the occurrence of a 15 S subunit component (FCV sediments at 170 S) encountered during the purification of FCV was described [11]. Both the subunit component and the whole virus possessed the same antigenic determinants and in addition, both homologous antisera exhibited virus neutralizing activities [12].

In the present report, the use of the 15 S subunit component in the preparation of a FCV vaccine was described. The vaccine was tested by immunizing cats which were later challenged with a virulent strain of FCV.

Materials and Method

Virus and cell culture

FCV and its accompanying 15 S subunit component were produced by infecting monolayers of feline embryonic fibroblasts of the FEA strain with the G-1 strain of FCV [8,15].

Virus and subunit purification

Virus purification and harvesting of the subviral component has been previously described [13]. Briefly, FCV was grown in FEA cells in leucine-free MEM with 1% fetal bovine serum.

A confluent monolayer in a 2.5-litre roller bottle was washed and the virus inoculated at a multiplicity of infection (m.o.i.) of 2 in 20 ml of medium containing 1 mCi of L-[^{3}H]leucine (specific activity 50 000 Ci/mol). After incubation for 16 h at 37°C the virus was harvested.

Cellular debris was removed by centrifugation at 10 000 rpm for 10 min, and an equal volume of cold saturated ammonium sulphate was added to the supernatant fluid. The mixture was maintained at 4°C for 3 h and then centrifuged at 5000 rpm for 5 min. The resulting precipitate was resuspended in Tris-saline (TS) buffer (100 mM NaCl, 10 mM Tris, 1 mM EDTA; pH 7.4) and a sample of 0.3 ml was layered on 4.5 ml 20–70% sucrose gradient which was then centrifuged in a Beckman SW 50.1 rotor at 45 000 rpm for 30 min. Two peaks of radioactivity were observed. The faster sedimenting component (P1) identified as the virus and the slower sedimenting virus subunit (P2) [13]. The latter was harvested for vaccine preparation. The molecular weight of the subunit component was however not determined in this study.

Vaccine preparation

Following harvesting and purification of the subviral component from FCV-infected cells, the purified subunits were pelleted in SW 50.1 rotor at 45000 rpm for 6 h, resuspended in 5 ml of TS and then sonicated. The vaccine was then filtered through a 25 nm Millipore filter to remove virus particles still trapped in the vaccine. The vaccine was aliquoted in 0.5 ml doses mixed with an equal volume of 1 : 10 dilution of Alhydrogel adjuvant (Miles Laboratories), and stored at −70°C until used.

Animals

Cats were obtained from the breeding colony of the University of Glasgow Department of Veterinary Pathology. 6 one-year-old cats were used, 4 of which were vaccinated (A, B, C and D) while the remaining two (X and Y) were kept as controls. All six cats were separately housed, and serologically screened for FCV antibodies before immunization.

Immunization

The vaccine was administered subcutaneously in a single dose in the neck region and a booster dose applied 4 weeks later. 10 days after the booster dose, the cats were challenged with 10^6 plaque-forming-units (pfu) of live FCV contained in 0.5 ml of Eagle's growth medium containing 2% calf serum.

Clinical manifestation of FCV disease

Following virus challenge, both immunized and control cats were examined daily for clinical signs of FCV disease which included a biphasic temperature rise, loss of condition, severe depression, inappetence and ulceration of the oral mucosa.

7 days after virus challenge two vaccinates and one control were killed; the others were killed 14 days post-challenge.

Collection of samples for virus isolation

Ante-mortem. Specimens were taken by moistening a sterile cotton wool swab in Eagle's minimal essential medium containing 2% fetal calf serum (EFC_2) and swabbing the appropriate area. The swab was broken into iced EFC_2 in a Bijou bottle.

Post-mortem. Samples of tissue were placed in 2 ml of EFC_2 cut into tiny pieces with sterile scissors before inoculation into cell culture.

Virus isolation

Virus isolation in tissue culture was done as earlier described [14]. Oropharyngeal swabs were taken just before virus challenge and one day after. Thereafter, samples were taken every other day until the cats were slaughtered. Samples of the tongue, tonsils, turbinates, trachea, lungs, kidneys, liver, spleen, retropharyngeal and bronchial lymph nodes were taken post-mortem for virus isolation.

Samples for bacteriology and histopathology

Both oropharyngeal swabs and lung tissues were taken for bacteriology but only the lung was taken for histopathology.

Plaque neutralization test

Equal volumes of a virus dilution calculated to give 60–70 pfu were mixed with serum dilutions. The mixture was allowed to react at 4°C for 1 h before inoculation of 0.2 ml of each serum/virus sample or control virus dilution on each of 2 plates of FEA monolayer cells per dilution. The standard plaque assay was then carried out [15]. The plaques were counted after 2 days. The titre of the serum was expressed as the reciprocal of the dilution which reduced the plaque count by 75% [14].

Results

Virus isolations

Pre-vaccination oropharyngeal swabs and similar swabs taken from all 6 cats immediately before virus challenge were negative for FCV. Table 1 shows the results of attempts to isolate virus from the oropharynx of both vaccinates and control cats examined every other day during the period of virus challenge.

All the cats began to excrete infectious virus within 24 h of inoculation. With the exception of one vaccinate (D) which excreted virus for 48 h, subsequent oropharyngeal swabs taken from other vaccinates proved negative. By contrast, virus was recovered from swabs of the controls up to the time of necropsy. The tonsils, considered to be the site for continued virus multiplication in carrier cats [17] were also found to be the most consistent source of virus isolation in this study.

Isolation of virus from post-mortem tissues of vaccinates all proved negative (Table 2). In the control cat (Y), necropsied on day 7, virus was recovered from the tongue, tonsils and the lungs, whereas only the tonsillar tissues were still found to be harbouring infectious virus in cat (X) necropsied on day 14.

Bacteriology

Swab samples taken for bacteriology failed to reveal the presence of pasteurellae,

TABLE 1

FCV isolation from oropharyngeal swabs (post-virus challenge)

Days	Control animals		Vaccinated animals			
	X	Y	A	B	C	D
0	–	–	–	–	–	–
1	+	+	+	+	+	+
3	+	+	–	–	–	+
5	+	+	–	–	–	–
7	+	+	–	–	–	–
9	+				–	–
11	+				–	–
13	+				–	–
14	+				–	–

+ = positive virus isolation; – = negative result.

Cats Y, A and B were necropsied on day 7; cats X, C and D were necropsied on day 14.

TABLE 2

FCV isolation from post-mortem tissues

Post-mortem tissues	Control cats		Vaccinated cats			
	PM7 (X)	PM14 (Y)	PM7		PM14	
			A	B	C	D
Tongue	+	-	-	-	-	-
Tonsil	+	+	-	-	-	-
Retropharyn. L.N.	-	-	-	-	-	-
Turbinates	-	-	-	-	-	-
Trachea	-	-	-	-	-	-
Lung	+	-	-	-	-	-
Spleen	-	-	-	-	-	-
Kidney	-	-	-	-	-	-
Bronchopharyn. L.N.	-	-	-	-	-	-

+ = positive virus isolation; - = no virus isolation.
PM7 and PM14 = cats necropsied on day 7 and 14.

bordetellae or β-haemolytic streptococci any of which could influence the course of calicivirus infection in cats. Also, none of the plates inoculated with materials from the lung yielded any growth.

Serology

Determination of the immune status of the 6 cats used in this study showed that all animals were serologically negative to FCV antibody prior to vaccination.

Prior to virus challenge, antibody titres of about 40 were observed in the vaccinates while there was no change in the immune status of the unvaccinated controls which still remained negative to FCV antibody. The antibody titre of the control cat (Y) necropsied on day 7 post-challenge rose slightly to 20, while an increase in titre to 40 was observed in the other control cat killed on day 14. The titre of the vaccinates rose only slightly to about 80, within the same period.

Clinical manifestations of FCV disease

The vaccinates remained clinically normal throughout the period after virus challenge whereas the controls exhibited characteristic symptoms of FCV infection including a biphasic temperature rise (beginning on day 1 and lasting 48 h and again on days 8 to 12), a progressive loss of condition, severe depression and inappetence. The tongue and the oral mucosa were severely inflamed but ulcers did not develop.

Post-mortem examination (cross pathology)

All tissues and organs examined post-mortem appeared normal except in one of the controls (Y) autopsied on day 7, where the lungs were purulent.

TABLE 3

FCV subunit vaccine: post-mortem lesions in vaccinated and control cats

	Lung pathology	Severity
Control animals		
X	Alveolitis, with focal accumulation of macrophages and polymorphs in alveolar spaces. Severe bronchiolitis and epithelial denudation of the bronchioles	+++
Y	Proliferative interstitial pneumonia with thickening of alveolar walls by mononuclear cellular infiltration. Hypertrophy and hyperplasia of type II pneumocytes. Severe alveolitis and bronchiolitis. Prominent peribronchial and perivascular lymphocytic infiltrations	++++
Vaccinated animals		
A	Occasional focus of alveolitis and bronchiolitis. Peribronchial and perivascular lymphoid foci	+
B	No abnormalities detected	-
C	Occasional focus of alveolitis and bronchiolitis	+
D	Alveolitis and bronchiolitis	+

+ = Severity of infection.

Histopathology

The histopathological findings on the lung tissues of both controls and vaccinates are presented in Table 3. No abnormalities were found in the lung of cat B, one of the vaccinates, while the others, A, C, D, showed a mild infection manifested by bronchiolitis and alveolitis.

Conversely, the unvaccinated cats exhibited lesions of severe infections and one of them (Y) killed 7 days post-challenge had proliferative interstitial pneumonia.

Discussion

The concept of subunit vaccines is a new development in antiviral research, starting with hepatitis B [20], human influenza [10] and, more recently, rabies subunit vaccines [4]. The results of the attempt made in this study to produce a subunit vaccine against feline calicivirus appeared encouraging.

One of the major attributes of the 15 S subunit particle is the ability of its homologous antiserum to neutralize FCV infection as effectively as the antiviral serum in neutralization tests [12]. When applied to in vivo situations as in this study, it was

found that none of the cats vaccinated with the subviral antigen developed the disease, whereas cats used as controls exhibited clinical and histopathological signs of disease (Table 3) and one was found to be pneumonic at post-mortem. Furthermore, unlike the vaccinates, both control cats continued to shed infective virus until slaughter (Table 2). These results are significant in the sense that despite the limited number of animals available in this study, the protective capability of the subunit vaccine against the calicivirus disease in domestic cats appeared to have been established.

Apart from its potency, the caliciviral subunit vaccine has the advantage of being inexpensive and this could be a valuable asset in terms of commercial preparation. Further experiments on the vaccine might profitably be done using filtered, crude, FCV-infected cell culture fluid to demonstrate that the expensive and time-consuming process of purification by centrifugation is unnecessary. However, it is likely that ultrafiltration would still be required as a final step in production.

An interesting question raised by the results of this study is to what extent the inactivated FCV vaccine described by Povey and Wilson [19] relied on its presumed content of 15 S subunit for its efficacy, in view of the fact that of the virus-specific components in viral harvests, the 15 S subunit contains ten times as much viral protein as is in whole virus [12]. It may be that by a process of filtration of the harvests, rather than chemical treatment, a superior immunogen is obtained. Further experiments are required to answer these questions.

Feline calicivirus, therefore, joins hepatitis B [20], influenza [10] and rabies [4] as examples of viruses from which a non-infectious subunit may be generated which acts as a vaccine against virulent virus challenge. The calicivirus subunit vaccine is important not only for the use to which it might be put in cats, but in the context of a possible model for the development of future vaccines for other small RNA viruses of animal and man.

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