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Attempted immunisation of cats against feline infectious peritonitis using canine coronavirus

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Specific pathogen free kittens were vaccinated with an unattenuated field isolate of canine coronavirus (CCV) either by aerosol or subcutaneously, and received boosting vaccinations four weeks later. Aerosolisation elicited a homologous virus-neutralising (VN) antibody response that increased steadily over a four-week period and levelled off one to two weeks after revaccination. The initial aerosolised dose produced an asymptomatic infection with excretion of CCV from the oropharynx up to eight days after vaccination; virus shedding was not detected, however, after the second inoculation. Cats vaccinated subcutaneously developed low VN antibody titres after the first CCV dose and experienced a strong anamnestic response after the second dose. Neutralising antibody titres then levelled off one to two weeks after revaccination at mean values somewhat lower than in cats vaccinated by aerosol. CCV was not isolated from the oropharynx after either subcutaneous dose. Four weeks after CCV boosting inoculations, vaccinated cats and sham-vaccinated control cats were divided into three subgroups and challenged by aerosol with the virulent UCD1 strain of feline infectious peritonitis virus (FIPV UCD1) at three different dosage levels. Five of six cats (including sham-vaccinated controls) given the lowest challenge dose showed no signs of disease, while all other cats developed lesions typical of feline infectious peritonitis (FIP). The five surviving cats developed FIP after subsequent challenge with a fivefold higher dose of FIPV. Thus heterotypic vaccination of cats with CCV did not provide effective protection against FIPV challenge.

FELINE infectious peritonitis virus (FIPV), a member of the family Coronaviridae, is an important pathogen of domestic and exotic cats (Barlough and Weiss 1983, Barlough and Stoddart 1986, Scott 1986, Pedersen 1987). It is the causative agent of a lethal, immunologically mediated vasculitis, feline infectious peritonitis (FIP), characterised by fibrinous peritonitis or pleuritis and formation of disseminated pyogranulomas. Current therapies, usually consisting of corticosteroids or other more potent cytoreductive drugs, are only palliative in nature and in most instances are ineffective at halting the relentlessly progressive course of the disease.

A safe and effective FIP vaccine has not yet been developed. Experiments thus far reported using various viruses within the FIPV antigenic cluster (Pedersen et al 1978) have not been successful in conferring uniformly protective immunity (Toma et al 1979, Woods and Pedersen 1979, Pedersen et al 1981, 1984, Pedersen and Black 1983, Barlough et al 1984b, 1985, Pedersen and Floyd 1985). Paradoxically, because of the immunopathological nature of the disease, vaccination with some feline coronaviruses has actually predisposed cats to the development of FIP and produced a more rapid and fulminating disease after FIPV challenge. Heterotypic vaccination using cross reactive coronaviruses (transmissible gastroenteritis virus [TGEV] of pigs, canine coronavirus [CCV], or human coronavirus 229E) has neither sensitised nor protected cats in most experiments. The mechanisms of sensitisation and immunity in FIP are incompletely understood at present, but it may be supposed that, as with many virus infections, a properly balanced cell-mediated immune response involving T lymphocytes, natural killer cells and activated macrophages is essential for effective host resistance.

In an earlier pilot study (Barlough et al 1984b), the authors demonstrated that CCV is capable of infecting cats and that antibodies elicited by multiple daily oronasal doses of the virus could cross react in a commercially available coronavirus antibody test using TGEV as target antigen. Vaccination with CCV produced no clinical signs of infection, did not result in excretion of detectable virus in faeces, and did not protect cats against FIPV challenge. The number of cats used in this first study was small, however, and only a large challenge dose of FIPV was investigated. A larger study using a greater number of cats was then undertaken, vaccinating by aerosol and subcutaneous

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routes, assaying for virus excretion from the oropharynx and challenging with three different doses of FIPV, all of which were smaller than the dose used in the pilot study. The results of this more comprehensive vaccine trial are the subject of the present communication.

Materials and methods

Animals

Eighteen 14-week-old specific pathogen free kittens (nine female, nine male) were purchased from Liberty Laboratories and housed singly in negative-pressure fibreglass isolation cages that were specially equipped for maximal air exchange. Cats from this commercial breeding colony are free of serum coronavirus antibodies, feline leukaemia virus and other feline virus infections, but are vaccinated against feline panleucopenia with an inactivated vaccine. Strict isolation procedures were followed in the care of animals throughout the experiment and, to minimise the possibility of virus contamination between groups, vaccinated and sham-vaccinated control cats were cared for on alternate days. Two adult coronavirus antibody-negative cats obtained previously from the same breeding colony were used as sentinel room controls.

Viruses and cells

A cloned stock of the Karbatsch isolate of CCV was propagated in canine A-72 cells as described previously (Barlough et al 1983b, 1984b). This virus was originally isolated in March 1978 from faeces of a dog with enteritis (L. E. Carmichael, Cornell University, personal communication). A virulent strain of FIPV (FIPV UCD1) (Pedersen 1976) was prepared as a liver homogenate (50 per cent concentration in culture medium) after passage through specific pathogen free cats. Homogenates were centrifuged at 200 *g* for 10 minutes at 4°C and the supernatants pooled and stored at −70°C for use as challenge inocula. Previous similar preparations (Weiss 1981) were determined to contain approximately 100 cat infective doses (ID100) ml^{-1}. Four concentrations of homogenate (25 per cent, 5 per cent, 2·5 per cent and 0·5 per cent, in Leibovitz's L-15 medium [Gibco]) were used in this study.

Serological assays

Virus neutralisation (VN) tests using CCV (Karbatsch) were performed essentially as described (Barlough et al 1983b), with only minor modifications. Coronavirus antibody titres were also determined in a computer-assisted, kinetics-based enzyme-linked immunosorbent assay (KELA), using TGEV as antigen, as described previously (Barlough et al 1983a, 1987).

Virus isolation

Oropharyngeal swab samples were eluted individually in 1·0 ml of L-15 medium and stored at −70°C. Upon thawing, 0·1 ml of each sample was added to quadruplicate wells of A-72 cells grown in 24-well plates (Costar). Cell monolayers were observed for seven days for development of the characteristic syncytial cytopathic effect of CCV (Karbatsch) (Barlough et al 1983b). Cytopathic effect was usually evident within two days of inoculation of cells with positive samples.

Experimental design

Specific pathogen free cats were initially divided into five experimental groups as shown in Table 1. On days 0 and 27, cats in groups 1 to 4 were vaccinated with either CCV (vaccinated cats) or uninfected A-72 cell culture fluid supernatant (sham-vaccinated control cats). Cats in group 1 were each given 5 ml of A-72 culture supernatant containing 2×10^7 TCID50 CCV by an aerosolisation procedure described previously (Weiss and Scott 1981, Barlough et al 1984b). Briefly, cats were confined within a plastic anaesthetic chamber (Searles Industries), and aerosolisation was performed with a fine-particle (under 0·5 μm) nebuliser (Hoechst-Roussel Pharmaceuticals) at 20 psi for 10 minutes. Cats remained in the nebulised fog for an additional 10 minutes before removal from the chamber. Cats in group 2 were inoculated by a single subcutaneous injection in the cervical region with 2 ml of culture supernatant containing 8×10^6 TCID50 CCV. Cats in groups 3 and 4 were sham-vaccinated with uninfected culture supernatant by the aerosol and subcutaneous routes, respectively. Cats in group 5 were maintained as sentinel room controls and were not exposed to CCV, FIPV or uninfected A-72 culture supernatants.

On day 55, four weeks after the second vaccination, cats in groups 1 to 4 were challenged with FIPV UCD1 by aerosol according to the scheme illustrated in Table 1. Three concentrations of liver homogenate were employed (25 per cent, 5 per cent and 0·5 per cent) and aerosolisation was performed as described for vaccination, using 5 ml of the appropriate FIPV preparation. The amount of virus delivered was calculated to be 250, 50 or 5 ID100, respectively, for each of the three homogenate concentrations. Cats that survived the lowest FIPV challenge dose were re-challenged on day 125 (10 weeks after the first challenge) with a 2·5 per cent liver homogenate preparation containing approximately 25 ID100.

All cats were monitored daily for clinical signs, and

TABLE 1: Results of challenge of CCV-vaccinated and sham-vaccinated cats with FIPV UCD1

Group	Group description	Number of cats per group	Subgroup	FIPV challenge dose (per cent concentration of liver homogenate)	Number of cats dying of FIP/ number of cats per subgroup	Mean survival time* (days after challenge ± SEM)
1	CCV-vaccinated (aerosol)	6	A	25	2/2	
			B	5	2/2	25·2 ± 3·1
			C	0·5 (2·5)†	2/2	
2	CCV-vaccinated (subcutaneous)	6	A	25	2/2	
			B	5	2/2	23·0 ± 3·1
			C	0·5 (2·5)	2/2‡	
3	Sham-vaccinated (aerosol)	3	A	25	1/1	
			B	5	1/1	
			C	0·5 (2·5)	1/1	23·3 ± 1·4
4	Sham-vaccinated (subcutaneous)	3	A	25	1/1	
			B	5	1/1	
			C	0·5 (2·5)	1/1	
5	Room controls§	2	—	—	—	—

* Interval between lethal FIPV challenge dose and death

† Five of six cats given the lowest FIPV challenge dose (0·5 per cent liver homogenate) on day 55 did not develop FIP. After a subsequent challenge on day 125 with 2·5 per cent liver homogenate, all five developed typical FIP. Mean survival times for these five cats were calculated using day 125 (rather than 55) as their day of lethal FIPV challenge

‡ One cat in this subgroup succumbed to FIP after receiving the lowest FIPV challenge dose

§ Not exposed to either CCV or FIPV

oropharyngeal swab samples for CCV isolation were collected at variable intervals (ranging from once daily to once weekly) during the vaccination portion of the experiment. Blood samples for coronavirus antibody detection were obtained once weekly by jugular venipuncture. Cats given FIPV were humanely killed when they became moribund by intracardiac administration of a euthanasia solution (T-61^{R}, National Laboratories), after initial anaesthesia with ketamine hydrochloride, and tissues were collected for histopathological examination in order to confirm the clinical diagnosis. Clinical observation of room control cats (group 5) was continued for several months after termination of the experiment.

Statistical methods

Differences in mean survival time after lethal FIPV challenge were tested for significance by the Mann-Whitney rank sum method for unpaired measurements (Snedecor and Cochran 1980).

Results

Response to challenge (Table 1)

All cats given the two larger doses of FIPV (subgroups A and B) developed clinical signs of FIP; the diagnosis was confirmed by histopathological examination of tissues after death. Five of six cats given the smallest challenge dose (0·5 per cent liver homogenate, subgroup C) remained healthy while the sixth, which had been vaccinated with CCV subcutaneously, succumbed to FIP. After a second challenge with a fivefold higher dose of FIPV (2·5 per cent liver homogenate) 10 weeks later, however, all five surviving cats experienced a typical FIP disease course. Mean survival times following lethal FIPV challenge were not significantly different among aerosol CCV-vaccinated, subcutaneous CCV-vaccinated, and sham-vaccinated groups of cats ($P>0·05$). Sentinel room control cats did not develop

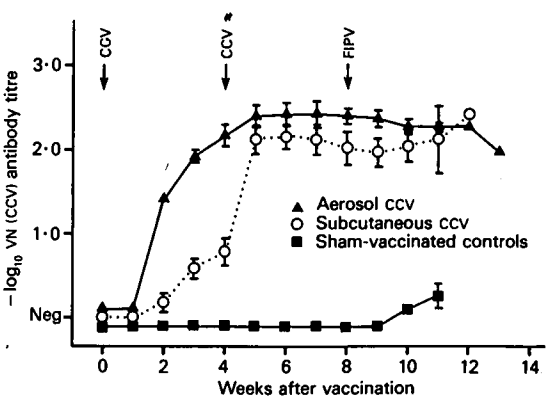

FIG 1: VN (CCV) antibody responses in CCV-vaccinated and sham-vaccinated cats (mean ± SEM). Arrows indicate inoculations with CCV (Karbatsch) and FIPV UCD1, the latter representing the day of lethal challenge. For the subgroup C cats that survived, the time interval between the first FIPV challenge and the second, lethal challenge is not shown, so that all data after challenge represent synchronised responses to FIPV. Aerosol CCV = group 1; subcutaneous CCV = group 2; sham-vaccinated controls = groups 3 and 4; sentinel room controls (group 5) not shown

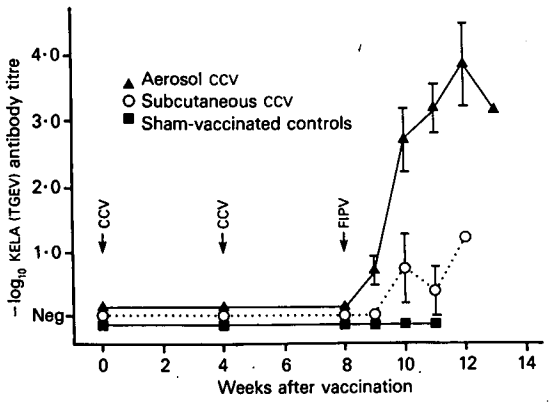

FIG 2: KELA (TGEV) antibody responses in CCV-vaccinated and sham-vaccinated cats (mean ± SEM). Arrows indicate inoculations with CCV (Karbatsch) and FIPV UCD1, the latter representing the day of lethal challenge. For the subgroup C cats that survived, the time interval between the first FIPV challenge and the second, lethal challenge is not shown, so that all data after challenge represent synchronised responses to FIPV. Aerosol CCV = group 1; subcutaneous CCV = group 2; sham-vaccinated controls = groups 3 and 4; sentinel room controls (group 5) not shown

signs of FIP and remained healthy throughout the course of the experiment.

Serological responses

Virus-neutralising antibody titres against CCV after vaccination and challenge are shown in Fig 1. Cats vaccinated with CCV by aerosol (group 1) developed VN antibody titres that were first detected two weeks after vaccination and that rose steadily during the succeeding two weeks. A further but less dramatic increase occurred after the second CCV vaccination, with titres levelling off at a mean value of 1/260. A heterotypic VN anamnestic response was not seen after FIPV challenge; instead, titre levels in individual cats remained stable until their death. Cats vaccinated with CCV by the subcutaneous route (group 2) developed extremely low VN titres that were first detected two weeks after vaccination. These titres rose only marginally during the succeeding two weeks but were boosted to high levels by the second vaccination, reaching a plateau at a mean value of 1/125. Neutralising antibody titres in individual cats remained relatively stable after FIPV challenge. Sham-vaccinated cats (groups 3 and 4) were consistently negative in CCV VN tests before FIPV challenge. Extremely low levels of heterotypic VN antibody were detected terminally in only the two subgroup C cats after the second, lethal challenge.

Cross-reacting antibodies to TGEV were not detected in any cat until after lethal FIPV challenge (Fig 2). A rapid and dramatic rise in KELA titres occurred in aerosol-vaccinated cats (group 1) after challenge, perhaps reflecting the production of non-neutralising antibody (Barlough et al 1984b). The two cats that survived the lowest FIPV challenge dose (subgroup C) did not develop KELA titres until after the second, lethal challenge. Antibodies to TGEV were detected in only two subcutaneously vaccinated cats (group 2) after challenge (the one surviving subgroup C cat developed KELA titres only after the second, lethal FIPV challenge). Several cats given subcutaneous inoculations developed elevated background reactivity in their serum as detected by KELA; such reactivity has been associated previously with humoral immune responses to extraneous, non-coronaviral components of cell culture medium (Barlough et al 1984a). All sham-vaccinated cats (groups 3 and 4) remained negative by KELA after FIPV challenge.

Sentinel room control cats were seronegative in both assays throughout the course of the experiment (data not shown).

Virus excretion

During the vaccination portion of the experiment, oropharyngeal swabs for CCV isolation were collected sequentially from four cats in the aerosol-vaccinated

TABLE 2: Oropharyngeal excretion of CCV by cats after CCV vaccination

		Experiment day																						
		First vaccination											Second vaccination											
Group	Cat	0*	1	2	3	4	6	8	11	14	18	22	27*	28	29	30	31	33	35	38	41	45	55
1†	UT5	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UU6	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UX1	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UZ3	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2‡	UU5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UZ2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

* CCV vaccinations
† Cats vaccinated by aerosol
‡ Cats vaccinated subcutaneously

group (group 1) and from two cats in the subcutaneously vaccinated group (group 2) (Table 2). All four cats from the aerosol group shed virus from the oropharynx up to eight days after the first vaccination; shedding did not occur, however, after the second CCV vaccination. The two cats in the subcutaneous group did not shed detectable amounts of CCV after either vaccination.

Discussion

The recognised antigenic cross reactivity between FIPV and CCV originally prompted the authors to examine the potential of CCV for protection of cats against virulent FIPV challenge. In an earlier pilot study (Barlough et al 1984b), two cats were vaccinated with CCV (Karbatsch) oronasally and challenged with a large dose (approximately 1000 ID_{100}) of FIPV UCD1 by aerosol. Both cats succumbed to FIP with a mean survival time of $25 \cdot 5 \pm 2 \cdot 5$ days, and sham-vaccinated control cats experienced a similar disease course (mean survival time $23 \cdot 5 \pm 1 \cdot 5$ days). Feline coronavirus antibody-sensitised control cats, however, died $8 \cdot 5 \pm 0 \cdot 5$ days after FIPV challenge ($P<0 \cdot 005$). It was therefore concluded that vaccination of cats with CCV did not sensitise them to develop accelerated FIP after subsequent FIPV exposure. The expanded study reported here was performed to evaluate more fully the potential of CCV as a heterotypic immunogen for FIP. Cats were vaccinated with CCV by aerosol to induce a potentially more protective mucosal immunity against aerosol challenge than the oronasal vaccination used in the pilot study. In addition, lower, graded challenge doses (5 to 250 ID_{100}) of FIPV were used. It was not possible, however, to demonstrate convincing protection against even the lowest FIPV challenge dose; thus it appears that vaccination of cats with CCV cannot confer protection against FIPV.

Although it initially seemed that five of six cats receiving the lowest FIPV dose (subgroup C) had resisted challenge, a second challenge with 25 ID_{100} of virus showed that they were not immune. The most likely explanation is that these five cats did not receive an infective dose of FIPV during the initial aerosol challenge. This view is supported by three observations: (i) the characteristic KELA response in the CCV-vaccinated cats and the terminal CCV VN response in the sham-vaccinated cats, both of which appeared only after the second, lethal FIPV challenge dose; (ii) the evolution of immune responses in the surviving sham-vaccinated cats, which was primary rather than anamnestic; and (iii) the absence of sensitisation in all five cats, as indicated by mean survival times (infection with FIPV UCD1 by the initial challenge dose would have sensitised the cats so that after the second FIPV UCD1 challenge the more rapid and fulminating FIP disease course would have ensued (Barlough et al 1984b).

The present experiment documents CCV shedding from cats for the first time. Cats vaccinated by aerosol were shown to excrete CCV from the oropharynx for up to eight days after inoculation; following revaccination with CCV, however, virus shedding did not occur, suggesting that the cats were immunised against CCV. Unfortunately this immunity was not heterotypic and did not extend to FIPV.

The VN antibody response elicited by the first CCV aerosol vaccination increased steadily over the succeeding four weeks in a manner suggesting in vivo amplification of the inoculated dose (Fig 1) (Mims 1982). This result also was obtained in the earlier pilot study and is now further supported by the pattern of CCV excretion from the oropharynx (Table 2). Considered together with the apparent absence of shedding following the boosting CCV vaccination, it would appear that limited replication of CCV occurred in the aerosol-vaccinated cats and that these cats were then to some degree immunised against subsequent CCV infection. By contrast, subcutaneous inoculation of cats with CCV elicited an initial, low level VN response that was boosted considerably by revaccination, data indicative of an anamnestic response to an inert immunogen (Mims 1982) and suggesting little or no replication of CCV in cats vaccinated subcutaneously.

Unlike the authors' earlier study, CCV-vaccination did not elicit cross-reacting antibodies to TGEV detectable by KELA. In the previous experiment, however, cats developed KELA titres only after repeated daily oronasal administration of CCV, not after the initial two doses. As described previously (Barlough et al 1984b), this phenomenon is attributed to two factors. First, administration of low doses of coronaviruses can result in production of only neutralising antibodies directed against the viral peplomer while higher doses can result in additional antibodies to non-peplomer determinants that are non-neutralising. Second, neutralising anti-peplomer antibody is detected by VN, but perhaps not by KELA due to loss or antigenic alteration of the fragile peplomers during TGEV antigen preparation. Cats in the present experiment may not have been given a dose of CCV sufficient to produce detectable non-peplomer antibody, and thus remained negative by KELA until after lethal FIPV challenge.

The dramatic anamnestic response in the aerosol-vaccinated cats suggests some priming of the immune response by non-peplomer determinants before challenge; the meagre KELA response of subcutaneously vaccinated cats after challenge may reflect a smaller degree of priming attributable to little or no CCV replication. As in the pilot study, sham-vaccinated cats remained negative by KELA even after

lethal FIPV challenge. The authors have often encountered the absence of a KELA response against TGEV in coronavirus-naive cats after experimental FIPV challenge, and it is probably due to a combination of the induction of a primary rather than anamnestic immune response, the swiftly fatal experimental disease course, immune complex formation and lesser sensitivity of the heterologous assay.

This study confirms the earlier findings that CCV-vaccinated cats are not sensitised to FIPV. Previous studies have shown that some cats with pre-existing coronavirus antibody, when challenged with FIPV, develop a more rapid and fulminating form of the disease than do seronegative cats similarly challenged (Toma et al 1979, Pedersen and Boyle 1980, Weiss et al 1980, Pedersen et al 1981, 1984, Weiss 1981, Weiss and Scott 1981, Pedersen and Black 1983, Barlough et al 1984b, 1985, Pedersen and Floyd 1985). Feline coronavirus strains appear to predominate as sensitising coronaviruses; neither TGEV, human coronavirus 229E nor CCV has produced significant sensitisation in experiments reported thus far. Sensitisation appears to be coronavirus strain-specific and dependent on the identity not only of the sensitising virus but also of the challenge strain. The sensitisation phenomenon has proved to be one of the major impediments to the development of a safe and effective FIP vaccine; thus it was hoped that a non-sensitising, cross-reactive coronavirus such as CCV might be able to provide immunity without sensitisation. To date, however, this strategy has not met with success.

The mechanisms governing immunity in FIP remain largely unidentified. Results from work carried out over the past decade suggest, however, that the humoral immune response is probably of minor significance and, when of a sensitising nature, may even be detrimental to the host. In other reports of FIP immunisation attempts and in more recent experiments performed in this laboratory, vaccinated cats have responded unpredictably and idiosyncratically to virulent FIPV challenge. Protection has often been an all-or-nothing phenomenon wherein some cats are not protected yet others are completely resistant to identical challenge doses administered in an identical manner. It is our opinion that further, very fundamental studies of virus-host interactions and the mechanisms governing immunity to FIPV must be performed before guidelines for a realistic immunisation strategy can be sketched out.

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