VIROLOGIC AND IMMUNOLOGIC ASPECTS OF FELINE INFECTIOUS PERITONITIS VIRUS INFECTION

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Summary

A number of feline coronavirus isolates have been characterized over the last few years. These isolates consist of what we have referred to as feline enteric coronaviruses (FECVs) and feline infectious peritonitis viruses (FIPVs). FECVs cause a transient enteritis in kittens but no systemic illness. FIPVs, in contrast, cause a systemic and usually fatal disease syndrome characterized either by an exudative serositis or a disseminated granulomatous disease. Although the diseases they cause are quite different, FECVs and FIPVs are antigenically and morphologically indistinguishable from each other. FECVs have a strict tropism for mature intestinal epithelial cells and do not appear to replicate in macrophages. In contrast, FIPVs, appear to spread rapidly from the intestinal mucosa and replicate in macrophages.

Experiments will be presented, and literature cited, that will allow us to make the following assumptions about the pathogenesis of FIPV infection: 1) FIPVs and FECVs represent a spectrum of viruses that differ only in infectivity (ability to evoke seroconversion following oral infection) and virulence (ability to cause FIP), 2) field isolates are generally nearer to FECVs in behavior than laboratory isolates made from animal passaged material, 3) immunity to FIPV appears to be of the premunition type and is maintained for as long as the infection persists in a reactivatable form, 4) strains of feline coronarviruses that do not cause systemic disease, such as FECVs or low virulence FIPVs, can actually sensitize cats to infection with virulent FIPV strains, 5) FeLV infection interferes with established FIP immunity and allows for the reactivation of disease in healthy carriers, 6) FIPV may be passaged from queen to kitten either *in utero* or during neonatal life, and 7) kittens infected by their mothers with FIPV do not usually develop FIP but become immune carriers of the virus for a period of 5-6 months; recovery from the carrier state is associated with a loss of premunition immunity.

Introduction

Feline Infectious Peritonitis (FIP) is a common infectious disease of cats characterized by either a severe exudative peritonitis and/or pleuritis or a widely disseminated granulomatous disease. FIP is caused by a coronavirus that is closely related to, and possibly a strain

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variation of, transmissible gastroenteritis virus (TGEV) of swine and canine coronavirus (CCV) (Horzinek et al., 1979). The pathogenesis of the disease is highly complex and involves incompletely understood interactions between host immunity and virus replication.

During the last 20 years since the discovery of its infectious and transmissible nature, almost 300 articles have been written on FIP. Each of these articles has contributed some new information about the clinical appearance of the disease, etiologic agent, or pathogenesis of infection. In spite of this plethora of information, a number of essential questions remain concerning the pathogenesis of the disease, possible difference among various field isolates of the virus, and nature of host immunity as it applies to disease and recovery.

The purpose of this article is to describe a series of experiments done in our laboratory or reported in the literature that provide possible answers to a number of questions regarding FIPV infection of domestic cats. These questions include: 1) do various isolates of FIP differ in infectivity and virulence, 2) does infection with one strain confer immunity against reinfection with the same or different strain, 3) does cellular immunity play an important role in disease protection, 4) can FIP exist as a latent or subclinical disease, 5) is there maternal transmission of FIPV from carrier queens to their kittens, and 6) does maternal transmission of FIPV to kittens lead to a state of immunity or disease? Following a presentation of these experiments, an attempt will be made to provide a unifying concept of pathogenesis and immunity.

Materials and Methods

Experimental Animals - Specific-pathogen-free (SPF) kittens were obtained from the breeding colony of the Feline Leukemia Research Laboratory, University of California, Davis. Animals were housed in federally approved experimental animal quarters of the Animal Resources Services, University of California, Davis.

Virus Strains - The derivation of FIPV-UCD1, (Pedersen et al., 1981a), FIPV-TN406 (Black, 1980), FIPV-79-1146 (McKeirnan et al., 1981; Pedersen et al., 1984b), and FIPV-Nor15 (Evermann et al., 1981) have already been described. FIPV-UCD2 was originally isolated from a one-year-old purebred Siamese with naturally occurring effusive FIP. Ascitic fluid from this animal was centrifuged, and the cell pellet was coculitvated with Fcwf-4 cells. Fcwf-4 are fetal feline lung cells that

have characteristics of macrophages (Jascobse-Geels and Horzinek, 1983). A characteristic cytopathic effect was seen after the second copassage. The virus was frozen down at the fifth cell passage; new inocula were started always from this material. FIPV-UCD3 was isolated from a three-year-old feline leukemia virus (FeLV)-positive domestic short-haired cat with naturally occurring effusive FIP. The manner of isolation was as described for FIPV-UCD2 with one exception; the resulting cultures were coinfected with FeLV. To remove the FeLV, culture supernatants containing both viruses were treated with goat anti-FeLV-gp70 serum (NIH 825-210) at a final dilution of 1:100. This was continued for five passages, at which time the virus was cloned in microtiter culture plates by limiting dilution. FIPV-UCD4 was isolated from a seven-year-old domestic short-haired cat with naturally occurring effusive FIP in the chest cavity. This cat was also coinfected with FeLV, and the virus was isolated in the same manner as FIPV-UCD3.

Animal Inoculation Studies - Cats infected with FIP-UCD2, -UCD3, and UCD 4 were given 1 ml of tissue culture fluids from Fcwf-4 cells infected with fifth-passage virus. The route of inoculation was either oronasal (1/2 ml orally and 1/2 ml intranasally) or intraperitoneal. Infection of cats with other strains of FIPV were conducted as previously reported (Black 1980; Evermann et al., 1981; Pedersen et al., 1981a, Pedersen et al., 1984b).

Persistent FeLV infections were induced in susceptible cats by giving 400,000 focus-forming units of FeLV-CT600 (Rasheed and Gardner, 1981) oronasally every other day for six days. In order to induce a persistent FeLV viremia, the cats were given 5 mg/kg of methylprednisolone intramusclarly at the time of the last inoculation (Pedersen et al., 1985; Rojko et al., 1982). Corticosteroid immunosuppression was induced with 5 mg/kg of methylprednisolone intramuscularly every seven days for three weeks (Pedersen et al., 1984).

Measurements of Cell-Mediated Immunity - Cell-mediated immunity to FIPV was measured in two ways: (1) by specific lymphocyte proliferation in response to FIPV antigen, and (2) by delayed-type hypersensitivity reaction to FIPV.

To measure specific lymphocyte stimulation, 12 ml of heparinized blood from an FIPV carrier or SPF cats were diluted 1:2 with Hank's buffered saline solution and layered onto 10 ml of lymphocyte separation

medium (Litton Bionetics) in sterile 50-ml conical tubes. The tubes were then centrifuged for 20 minutes at 400 x g at room temperature. The lymphocyte-rich fraction was removed and washed twice with Hank's buffered saline solution. Cells were resuspended in RPMI medium containing 2 x 10^6 cells/ml. Fifty microliters of the peripheral blood lymphocyte suspension were placed in each of 12 wells in a 96-well microliter plate (1 x 10^5 cells/well). Fifty microliters of tissue culture medium were added to each of three wells (negative controls), 50 ul of tissue culture medium containing 1 ug of living gradient purified FIPV-UCD1 were added to each of three wells (low dose of specific antigen), and 50 ul of tissue culture medium containing 10 ug of gradient-purified FIPV were added to each of three wells (high dose of specific antigen). The plates were incubated at 37°C in a humidified chamber containing 10% CO_2. Ten microliters of tissue culture medium containing 2 uCi of ^{3}H-methylthymidine were added to each well on the third day. Cell harvesting was done on Day 4 using a Flow Mash Cell Harvester (Flow Laboratories). Cells were harvested onto filter paper disks by flushing the harvester with absolute methanol. Disks were allowed to dry at room temperature and were placed into scintillation vials containing 5 ml of PCS/xylene (2:1) cocktail. Vials were stored at 4°C overnight and were counted on a beta scintillation counter the following day.

The delayed-type hypersensitivity reactions were evoked as follows: Cats were sedated with ketamine, and 0.1 ml (50 ug) of purified, sonically disrupted FIPV-UCD1 was injected intramucosally into the outer surface of the nicitiating membrane of the right eye. Saline (0.1 ml) was injected into the left eye. The eyes were examined at 12-hour intervals for signs of squinting, swelling, redness, blistering, or edema.

Serology -Antibodies to FIPV were measured by indirect fluorescent antibody assay using FIPV-UCD1-infected Fcwf-4 cells as a substrate (Pedersen et., 1981a; Pedersen 1976a).

Results

Infectivity and Virulence of Various FIPV Isolates - We have observed a great difference in the infectivity and virulence of various isolates of FIPV that have been studied in our laboratory and reported in the literature. Infectivity refers to the ability of a given dose of

virus to infect a cat as evidenced by the induction of serum antibodies, while virulence refers to the ability of an isolate to cause disease, i.e. FIP. The infectivity and virulence of 7 different FIPV isolates has been presented in Table I. It is apparent from experimental challenge-exposure studies that each isolate of FIPV differs somewhat from the other, and represent a spectrum in regards to infectivity and virulence.

Table I

Infectivity and Virulence (FIP-inducing Capacity) of a Number of Different FIPV Isolates

Strain	Route of Infection	Infectivity (Seroconversion)	Numbers of Cats That Died from FIP	Number of Cats That Remained Healthy	Reference Source of Data
FPV-UCD1	Oral	4/15	3/15	12/15	Pedersen, et al., 1981
	Intratracheal	7/10	6/10	4/10	
	Intraperitoneal	4/4	4/4	0/4	
FIPV-UCD2	Oronasal	5/5	0/5	5/5	Personal
	Intraperitoneal	5/5	0/5	5/5	Observation
FIPV-UCD3	Oronasal	4/4	0/4	4/4	Personal
	Intraperitoneal	5/5	2/5	3/5	Observation
FIPV-UCD4	Oronasal	4/4	0/4	4/4	Personal
	Intraperitoneal	8/8	3/8	5/8	Observation
FIPV-TN406	Oral	5/5	4/5	1/5	Black, 1980
FIPV-Nor-15	Oral	NT[a]	22/24	2/24	Evermann, et al., 1981
	Intravenous	NT	3/3	0/3	
	Intramuscular	NT	2/3	1/3	
	Intraperitoneal	NT	3/3	0/3	
FIPV-79-1146	Oronasal	3/3	3/3	0/3	Pedersen et al., 1984a,
	Intraperitoneal	4/4	4/4	0/4	Personal Observation

[a] NT = Not Tested

For instance, FIPV-Nor15 and FIPV-79-1146 are highly infectious and highly virulent. Seven out of 7 cats that were tested for antibodies seroconverted after challenge-exposure, while 37/40 cats in the two groups developed FIP and died. FIPV-UCD2 is at the opposite end of the spectrum; this isolate is highly infectious (10/10 cats infected seroconverted) but none of the infected cats developed any sign of FIP The remaining isolates were intermediate between these two extremes.

Infectivity and virulence also vary greatly between isolates depending on the route of inoculation (Table I). Highly infectious and virulent strains such as FIPV-79-1146 and -Nor15 appear equally infectious and virulent by oral and parenteral routes of challenge-exposure. Isolates such as FIPV-UCD3 and -UCD4, however, are equally infectious by oral and intraperitoneal routes but are much more virulent when given orally than intraperitoneally (Table I). FIPV-UCD1, which is relatively virulent, is less infectious by oral than parenteral routes. FIPV-UCD2 is highly infectious by both oral and intraperitoneal routes but has lost all virulence. Virulence and infectivity, therefore, are factors that operate independently of each other.

Autologous and Homologous Immunity Studies - As a result of experimental challenge-exposure studies, we were able to obtain a large number of kittens that had seroconverted after infection with various FIPV isolates without developing FIP. We were interested in seeing whether these cats were immune to reinfection with the same (autologous) isolate of FIPV or with different (homologous) isolates. Cats given FIPV-UCD2 either oronasally or intraperitoneally were resistant to reinfection with FIPV-UCD2 (Table II). They were, however, all susceptible to infection with FIPV-UCD1 and FIPV-79-1146 (Table III). In fact, the disease that they developed was frequently more acute and severe than that normally seen in susceptible specific pathogen free seronegative kittens. These cats appeared to be sensitized in the same manner, therefore, as has been described previously for cats with pre-existing homologous coronavirus immunity (Pedersen and Boyle, 1980; Pedersen et al., 1984b; Weiss and Scott, 1981b). Infection with FIPV-UCD2 was analagous, therefore, to infection with feline enteric coronavirus (FECV) (Pedersen et al., 1981b). FECVs are morphologically and antigenically indistinguishable from FIPV but do not cause FIP (Boyle et al., 1984; Pedersen, 1983a; Pedersen et al., 1984a; Pedersen et al., 1981b).

Three of four cats that had been infected primarily with FIPV-UCD3 were resistant to challenge exposure with the same strain (Table II). The greatest surprise came when FIPV-UCD3-recovered cats were challenge-exposed intraperitoneally with FIPV-UCD1. Most of the FIPV-UCD3-recovered cats were solidly immune to a dose of FIPV-UCD1 that was

Table II

FIP Occurrence in Cats Infected Initially with FIPV-UCD2, FIPV-UCD3, or FIPV-UCD4 and Challenge-Exposed Two to Four Months Later with the Same Strain

Strain and Route of Exposure Used for Primary Infection	Strain Used for Intraperitoneal Challenge-Exposure	Clinical Outcome		
		Accelerated FIP	Classic FIP	No Disease
FIPV-UCD2				
Oronasal	FIPV-UCD2	0/2	0/2	2/2
Intraperitoneal		0/1	0/1	1/1
FIPV-UCD3				
Oronasal	FIPV-UCD3	0/2	0/2	2/2
Intraperitoneal		0/1	0/1	1/1
FIPV-UCD4				
Oronasal	FIPV-UCD4	0/1	1/1	0/1
Intraperitoneal		0/1	0/1	1/1

Table III

Disease Course of Cats That Were Challenge-Exposed with Highly Virulent Strains of FIPV After an Asymptomatic Primary Infection with FIPV-UCD2, FIPV-UCD3, or FIPV-UCD4

FIPV Strain Used for Primary Infection	Route of Primary Infection	Strain of FIPV Used for Challenge Exposure	Number That Died from Accelerated FIP[a]	Number That Died from Classic FIP[b]	Number That Did Not Develop Illness
FIPV-UCD2	Oronasal	FIPV-UCD1	1/3	2/3	0/3
	Intraperitoneal	FIPV-UCD1	2/2	0/2	0/2
	Intraperitoneal	FIPV-79-1146	0/1	1/1	0/1
FIPV-UCD3	Oronasal	FIPV-UCD1	0/3	0/3	3/3
	Intraperitoneal	FIPV-UCD1	1/1	0/1	0/1
FIPV-UCD4	Oronasal	FIPV-UCD1	1/2	1/2	0/2
	Intraperitoneal	FIPV-UCD1	1/2	0/2	1/2
	Oronasal	FIPV-79-1146	1/2	1/2	0/2
	Intraperitoneal	FIPV-79-1146	0/1	1/1	0/1

[a] Accelerated FIP: Fever within 24 to 48 hours after challenge exposure, with a clinical course usually ending in death in 7 to 14 days

[b] Classical FIP: Fever occurs from 7 to 14 days after exposure, with a clinical course usually longer than 14 days

uniformly lethal to cats that had been primarily infected with FIPV-UCD2 (Table III).

Cats that had been primarily infected with FIPV-UCD4 showed a variable resistance to reinfection with FIPV-UCD4 (Table II). A similar pattern of poor resistance was seen against FIPV-UCD1. Only one of seven FIPV-UCD4-recovered cats was immune; three of seven developed accelerated disease, and three of seven developed conventional FIP (Table III).

It was concluded from this study that cats infected with FIPV-UCD2 neither became ill nor developed protective immunity against challenge exposure with more virulent strains of FIPV. FIPV-UCD3-recovered cats differed from FIPV-UCD2-recovered cats because they tended to be immune to challenge exposure with virulent FIPV-UCD1. FIPV-UCD4 was intermediate to FIPV-UCD2 and FIPV-UCD3 in its virulence. It caused a lower incidence of FIP than did FIPV-UCD3, but similarly to FIPV-UCD2, it did not induce good protective immunity against the more virulent FIPV-UCD1.

The Nature of FIPV Immunity - A number of studies done over the last decade have dealt directly with the nature of immunity to FIPV (Horzinek and Osterhaus, 1979; Pedersen and Boyle, 1980; Pedersen and Black, 1983; Weiss and Scott, 1981b). Immunity to the virus is apparently not humorally mediated (Pedersen and Black, 1983). In fact, humoral immunity by itself can enhance rather than protect against experimental infection (Pedersen and Boyle, 1980; Pedersen and Black, 1983; Weiss and Scott, 1981b). Cats that are infected with FECV develop antibodies that strongly cross-react with FIPV strains; and, in fact, FECV antibodies will even neutralize FIPV in vitro (Boyle et al., 1984; Pedersen et al., 1984a). If cats that have been infected with FECVs are challenge-exposed with FIPV, they will have a higher incidence of FIP, clinical signs will appear more rapidly, and the disease course will be more fulminating (Pedersen et al., 1984b; Pedersen et al., 1981a). The same phenomenon occurs when non-FIP-inducing variants of FIPV are used for preimmunization. A non-FIP-inducing variant of the TN406 strain of FIPV was selected by high passage in cell culture (Pedersen and Black, 1983). This high-passaged variant strain induced humoral immunity in cats, but the immunity was actually immunoenhancing to FIPV-UCD1 infection (Pedersen and Black, 1983). The same phenomenon occurred with the 79-1683 strain of FECV (Pedersen et al., 1984b). The UCD2 strain of FIPV is another example of a nonvirulent immunoenhancing strain of FIPV that

was derived from a virulent one. FIPV-UCD2 behaved like FIPV-TN406 (high passage) (Pedersen and Black, 1983), and these two strains in turn behaved like FECV strains such as UCD and 79-1683 (Pedersen et al., 1984b; Pedersen et al., 1981b). The ease with which non-FIP-inducing strains of FIPV can be isolated from cultures of virulent virus lends credence to the postulate that FIPVs and FECVs are merely virulence mutants of each other.

If humoral immunity does not protect cats against virulent strains of FIPV, what then is the nature of FIPV immunity? It has been postulated, that immunity to FIPV is largely cell mediated (Pedersen and Black, 1983). Reasons for this assumption include the following: 1) The noneffusive form of FIP resembles tuberculosis and deep mycotic infection (coccidioidomycosis, blastomycosis, and histoplasmosis) of humans and animals; immunity to these infections is known to involve mainly cellular mechanisms, 2) the clinical incidence of FIP can be increased greatly by concurrent FeLV infection, and FeLV infection is a potent suppressant of cellular immunity and of T-cell-mediated humoral immunity (Hardy, 1982)., 3) immunity to FIP cannot be transferred passively with hyperimmune serum, even if the serum contains virus-neutralizing antibodies (Pedersen and Boyle, 1980; Pedersen and Black, 1983), and 4) cats are known now to carry FIPV as a latent or sequestered infection, and this infection can be reactivated by infecting the immune carriers with FeLV. A carrier state of this type is common in microbial infections where cellular immunity is known to be the primary protective mechanism. Immunity is sustained in these situations by virtue of the persistent infection and its effects on the immune system.

Although experimental observations seem to indicate that cellular immunity is involved in FIPV protection, there has been no direct evidence that healthy FIPV-infected cats demonstrate any of the typical manifestations of specific cellular immunity. In experiments in our laboratory, SPF cats that had been infected previously with FIPV-UCD2, FIPV-UCD3, or FIPV-UCD4, and then FIPV-UCD1, were tested for cellular immunity. Many of these cats probably harbored FIPV, because a cohort group of these animals developed FIP when they were subsequently infected with FeLV (see Table IV). The tests that were used to detect cellular immunity were of two types: 1) delayed hypersensitivity reactions evoked by injecting purified and sonicated FIPV intramucosally into the third

eyelid, and 2) lymphocyte stimulation to FIPV antigen. Using these tests, specific delayed-type hypersensitivity reactions were demonstrated in the third eyelids of four of eight FIPV-recovered cats (data not shown). Specific lymphocyte blastogenesis to FIPV antigen was observed in peripheral blood lymphocyte cultures from 9 of 17 cats, albeit at low levels (Fig. 1). Four of the 9 lymphocyte positive cats were the same animals that had a delayed hypersensitivity reaction to FIPV antigen. Although a specific cell-mediated immune reaction of one type or the other was seen only in 9 of 17 animals, we felt that cell-mediated immunity was involved somehow in containing the FIPV infection in all of the animals. Cell-mediated immunity is notoriously hard to test in cats compared to humans and guinea pigs.

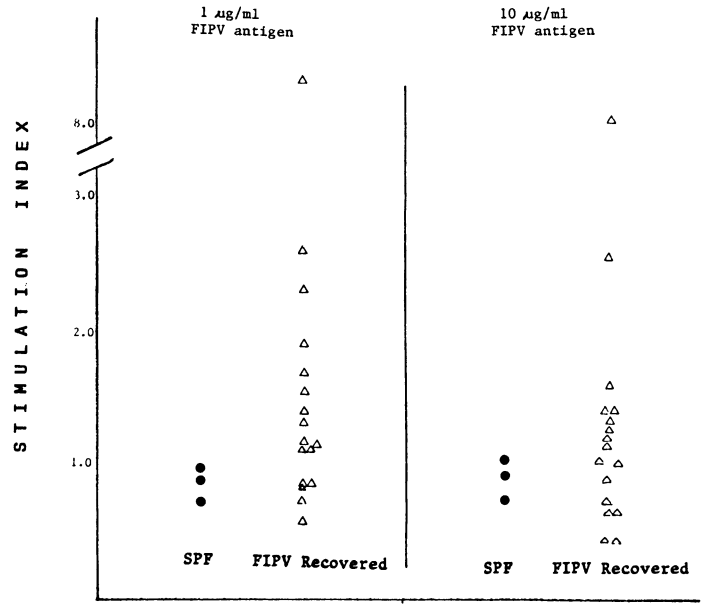

Fig. 1 - The stimulation of cultured peripheral blood mononuclear cells from FIPV recovered and specific pathogen free cats by live FIPV. Mononuclear cell cultures were exposed to either 1 or 10 ug/ml of FIPV in the culture supernatant. The stimulation index was the ratio of uptake of radioactively labeled thymidine by antigen stimulated compared to antigen unstimulated cell cultures.

Latent FIPV Infections - We questioned whether kittens that survived FIPV challenge-exposure still carried FIPV in their bodies in the form of a latent or subclinical infection. The similarity between FIPV induced disease and other granulomatous infections such as tuberculosis of man and animals suggested that this might be the case. The question of persistent virus infection in FIPV-recovered cats was answered by taking

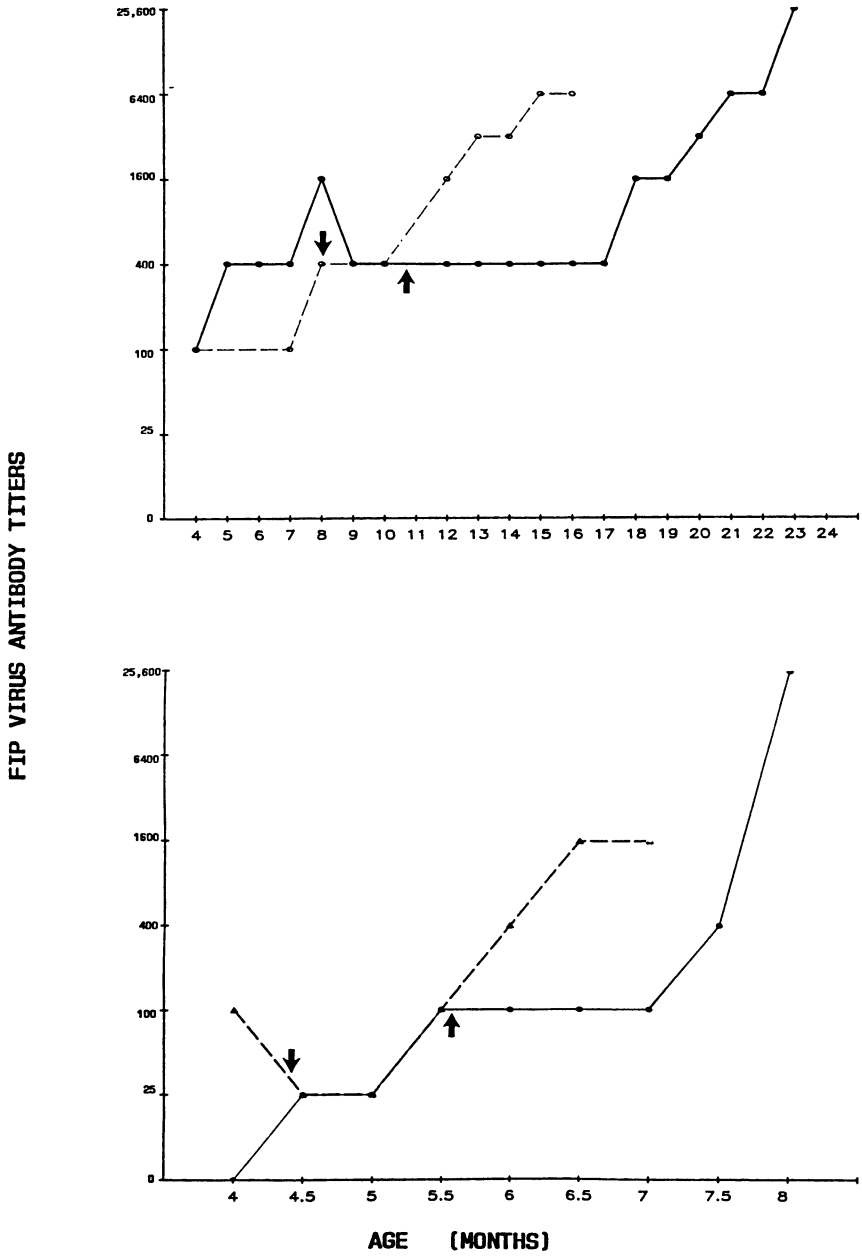

Fig. 2 - FIPV antibody titers of 4 cats that were experimentally infected with FeLV by natural contact with carrier cats. The cats became persistently FeLV infected at the time indicated by the arrows. FIPV antibody titers present at the start of FeLV exposure (3 months of age) reflected exposure to a coronavirus prior to the time they were placed on experiment. Antibody titers to FIPV rose dramatically several weeks or months after FeLV infection. All of the cats were euthanitized because of advanced FIP at the time of the last titer determination. Actual clinical signs of FIP were not noticeable until the last month or so of life.

Table IV.

Reactivation of Clinical FIP by Superimposed FeLV Infection in FIPV-Recovered Cats

Cat Number	Previous FIPV Exposure	Experimental Treatment	Clinical Outcome (weeks after treatment)
2017	FIPV-UCD3, FIPV-UCD1	Methylprednisolone	Healthy
3877	FIPV-UCD3, FIPV-UCD1	Methylprednisolone	Healthy
3880	FIPV-UCD3, FIPV-UCD1	Methylprednisolone	Healthy
3881	FIPV-UCD2, FIPV-UCD1	Methylprednisolone	Healthy
3919	FIPV-UCD3, FIPV-UCD1	CT600-FeLV and methylprednisolone	Effusive FIP (16)
2153	FIPV-TN406, FIPV-UCD1	CT600-FeLV and methylprednisolone	Effusive FIP (6)
2007	FIPV-UCD3, FIPV-UCD1	CT600-FeLV and methylprednisolone	Noneffusive (8)
3892	FIPV-UCD3, FIPV-UCD1	CT600-FeLV and methylprednisolone	Effusive (8)
2005	FIPV-UCD2, FIPV-UCD1	CT600-FeLV and methylprednisolone	Effusive (9)
1253	FIPV-UCD2, FIPV-UCD1	CT600-FeLV and methylprednisolone	Effusive (7)
2785	FIPV-UCD3, FIPV-UCD1 FIPV-79-1146	CT600-FeLV and methylprednisolone	Healthy

advantage of an interesting relationship between FIPV and FeLV infections. It is well knowen that FeLV infection is a cofactor for FIP (Cotter et al., 1975). About 10-40% of naturally induced cases of FIP occur in cats coinfected with FeLV. We have confirmed this relationship in our own experimental studies on FeLV. Among 500 or more cats that we have experimentally infected with FeLV by contactexposure with carrier cats, 35 died of FIP several weeks or months of the time that they became FeLV viremic. Only one cat died of FIP among littermates that were not exposed to FeLV. Figure 2 shows the serologic response to FIPV in several of these cats. Antibody levels to FIPV began to rise progressively after the cats became viremic for FeLV and reached high levels prior to death from FIP. How did these cats develop FIP? There were only 2 possibilities: 1) they became exposed to FIPV after they had become FeLV infected and the immunosuppressive effects of FeLV infection prevented them from recovering, or 2) they had a latent or subclinical FIPV infection in their body prior to the time they were FeLV infected and the immunosuppressive effects of FeLV infection caused it to be reactivated. The fact that the cats came from a cattery where FIPV was known to be endemic, and carried coronavirus antibodies prior to FeLV exposure, supported the latter hypothesis.

The answer to whether FIPV infection occurred before or after FeLV infectiion was obtained by the following experiment. A group of specific pathogen free kittens were infected with FIPV-UCD3 and -UCD4. Cats that failed to develop FIP after this infection were then reinfected with more virulent FIPV-UCD1. Cats that survived the second challenge-exposure were then experimentally inoculated with FeLV. The cats were kept in strict isolation during all of the experiment so there was no possible sources for extraneous FIPV or FeLV infection. To insure that the cats

would become persistently infected with FeLV, the FeLV challenge exposure was augmented with methylprednisolone (Rojko et al., 1982). To control for the effects of the methylprednisolone, a second group of FIPV-recovered cats was given methylprednisolone alone (weekly for three weeks) (Pedersen et al., 1984c). The four FIPV-recovered cats that were given methylprednisolone alone remained healthy over the three-month observation period (Table IV). In contrast, six of seven cats treated with methylpredinoslone and infected with FeLV developed classic effusive or noneffusive FIP within 6 to 16 weeks and died (Table IV). It was concluded, therefore, that many FIPV-recovered cats were indeed "immune carriers" of FIPV. This asymptomatic carrier state could be reversed readily by the immunosuppressive effects of FeLV infection but not with methylprednisolone.

Table V

Serum Antibodies in Kittens Born to FIPV-Recovered SPF Queens

Queen Number	FIPV Strains Queens Exposed To	Kitten Number	IFA Coronavirus Antibody Titer						
			Prenursing	Postnursing	1 Week Old	2 Weeks Old	4 Weeks Old	6 Weeks Old	8 Weeks Old
2283	UCD2/UCD1	2152	NT[a]	125	>625	625	625	25	625
		2153	NT	625	125	625	625	25	625
3838	UCD2/UCD1	2051	NT	125	625	25	25	5	5
		2052	NT	125	625	25	25	25	5
3822	UCD3/UCD1	2291	NT	5	5	0	5	0	0
		2297	NT	>625	>625	625	125	125	125
		2020	NT	625	>625	625	125	125	125
		Sentinel	NT	0	0	0	0	0	25
		Sentinel	NT	0	0	0	0	5	125
3822	UCD3/UCD1	2022	NT	0	125	625	25	625	625
		2023	NT	125	125	125	25	125	125
		Sentinel	NT	0	0	0	0	5	25
2006	UCD3/UCD1	2053	0	125	625	125	25	25	25
		2055	0	125	625	25	625	125	125
		2057	0	625	625	625	125	25	25
3863	UCD4/UCD1	2069	NT	125	625	625	125	125	125
		2070	NT	625	625	125	125	25	25
		2071	NT	125	125	125	125	25	125

[a]NT = not tested

Maternal Transmission of FIPV from Carrier Queens to Kittens - kittens born to FIPV-recovered queens were bled at one to two-week intervals from birth onward. The kittens acquired maternal antibodies to FIPV within the first day or so of life (Table V). The titer of these antibodies decreased during the first five weeks, after which two patterns were seen. In most of the kittens, the FIPV antibody titers reached their lowest levels by the fourth through sixth week and then began to increase. In the other kittens, the maternal antibodies declined and remained at a low level. Three sentinel SPF kittens that

were fostered onto these queens shortly after parturition developed antibodies four to eight weeks later (Table V). This pattern of maternal antibody acquisition and decline was identical to that previously described for kittens born into FIPV and FECV-endemic catteries (Pedersen 1976a; Pedersen et al., 1981b).

The obvious explanation for the rise in antibody titer that occurred from four to eight weeks of life was that the kittens were actively responding to naturally acquired infection. Because the kittens and queens were kept in strict isolation from other cats, the source of the infection had to be the queen. Was the infection acquired while the kittens were still in utero, or was it horizontally transmitted after birth? Horizontal transmission did occur, as witnessed by the sero-conversion of the sentinel kittens. It was possible, however, that the sentinel kittens were infected by the kittens rather than by the queen. This still left the possibility that the kittens were infected in utero and that they actively shed the virus after birth. It must also be noted that not all of the queens appeared to be infectious. In at least one litter of kittens, the maternal antibody titer waned and never did increase.

Immunity to FIP in Kittens Born to FIPV Recovered Queens - Kittens infected with FIP were challenge-exposed with virulent FIPV-UCD1 or FIPV-79- 1146 at either 8-10 or 22 weeks of age. Only 1 kitten in the 8-10 week age-group developed FIP following challenge-exposure. This kitten recovered completely, however, after manifesting clinical signs for 2 weeks. Kittens that were 22 weeks of age at the time of challenge-exposure did not fare nearly as well. Three out of 5 kittens developed FIP and died following challenge-exposure and the remaining 2 kittens were never visibly ill. Fevers in the 3 kittens that died appeared 3, 5, and 7 days, respectively, following challenge-exposure. This was shorter than the 7-14 day period from infection to initial fever that has been observed in cats with no previous exposure but longer than the 2-3 day period observed with previously sensitized cats (Pedersen and Boyle, 1980). It appeared that immunity to FIPV in previously recovered cats was finite in duration, waning after 5 months or so. Was it possible that the kittens remained immune to FIPV only for as long as they still retained the virus in their bodies as a latent or subclinical infection?

To see whether FIPV was lost from the body at the same time as immunity was waning, we infected a group of cats with FeLV that had recovered from initial FIPV challenge-exposure at varying times prior to the study. Six out of 7 of the cats that had been initially exposed to

Table VI

The Incidence of FIP in Cats that Survived Challenge-Exposure with Virulent FIPV and were Later Experimentally Infected with Virulent FeLV

Cat number	Days After First Exposure to Virulent FIPV Cats challenge-exposed to FeLV	Clinical Outcome After FeLV Infection *
2153	14	Effusive FIP
2005	18	Effusive FIP
1253	90	Effusive FIP
2785	90	Healthy
2007	90	Non-effusive FIP
3892	120	Effusive FIP
3919	127	Effusive FIP
2017	210	Healthy
3877	270	Healthy
3880	270	Healthy
3881	270	Healthy

* All cats except for #2785 developed an infection that persisted until death from FIP or termination of the experiment 1 year after exposure.

FIPV from 14 to 127 days prior to being infected with FeLV developed FIP and died (Table VI). In contrast, all 4 cats that had been infected with FIPV 7 or more months earlier survived. We interpreted these findings in 2 possible ways: 1) immunity to latent or subclinical FIPV infection became more difficult to abrogate with FeLV-induced immunosuppression as time passed, or 2) FIPV was cleared from the body of most cats after 4-6 months. The fact that FIP immunity in maternally infected kittens tended to wane after 4 months led us to favor the second possibility.

Discussion

Studies of various isolates of FIPVs and FECVs, along with previous work, have given us many new insights into the pathogenesis of FIP and the relationship that various feline coronaviruses have with each other. FECVs are on the low end of the virulence spectrum. They are highly infectious but generally do not cause FIP (Pedersen et al., 1981b;

Pedersen and Black, 1983; Pedersen, 1983a), or if they do, it is at a very low rate. FECVs are primary pathogens of the intestinal mucosa, and in kittens they cause enteritis that is usually mild and self-limiting (Pedersen et al., 1981b; Pedersen et al., 1981b; Pedersen 1983a). Occasionally, however, they can cause a more severe and even fatal enteritis (McKeirnan et al., 1981; Pedersen 1983a). The enteric coronaviruses apparently do not invade much beyond the regional lymphoid tissues of the oropharynx and intestinal tract (Pedersen et al., 1984b; Pedersen et al., 1981b). Even when seen in these regional lymphoid aggregates, there is no reason to believe that they replicate there to any extent (Pedersen et al., 1984b; Pedersen et al., 1981b). Following recovery from FECV infection, many cats remain "immune carriers" of FECV, or they lose and regain the infection at cyclic intervals throughout their lives (Pedersen et al., 1981b). Carrier cats shed the FECVs in the feces (Pedersen et al., 1984b; Pedersen et al., 1981b). FIPVs differ from FECVs in their ability to escape from the confines of the intestinal tract. Once outside of the intestinal tract, they replicate in distant reticuloendothelial tissue and induce the diseases known as effusive and noneffusive FIP (Pedersen, 1983b). This is a major difference between FECVs and FIPVs; FIPV replicates mainly in intestinal mucosal cells (Hayashi et al., 1978; Hayashi et al., 1982). Not only do macrophages provide a good substrate for FIPV growth (Jacobse-Geels and Horzinek, 1983), but they also are involved with the dissemination of the virus throughout the body (Weiss and Scott, 1981a). The factor or factors within FIPVs that confer this great potential for invasiveness and replication in macrophages is unknown. It does not appear, however, to result from major differences in the morphology or protein makeup of FIPVs compared with that of FECVs (Boyle et al., 1984). Whatever this virulence factor is, it seems to be possessed to radically different degrees by various strains of FIPV. The most virulent (FIP-inducing) strains of FIPV cause disease in almost all oronasally inoculated cats. FIPV-79-1146 (Pedersen et al., 1984b) and FIPV-Nor15 (Evermann et al., 1981) are examples of such highly virulent strains. Highly virulent strains do not appear to exist in great numbers in nature, though. Only a few naturally occurring outbreaks of FIP have been severe enough in

morbidity and mortality to suggest infection with high virulence FIP-inducing strains. Even in outbreaks of great severity, there is a tendency for the disease to subside or even disappear after six months to a year. Strains such as FIPV-UCD1 are in the middle of the virulence spectrum. If enough of this virus is given orally over a long period of time, a percentage of cats will develop FIP (Pedersen et al, 1981a). At the low-virulence end of the spectrum are such strains of FIPV as UCD3 and UCD4. These strains, especially when given oronasally, infrequently cause FIP. The spectrum of virulence from non-FIP-inducing to highly FIP-inducing would appear like this: FECVs (UCD and 79-1683), FIPV-UCD2, FIPV-UCD4, FIPV-UCD3, FIPV-UCD1, FIPV-TN406, FIPV-79-1146, and FIPV-Nor15. Except for FECV-UCD, all of these strains will grow in tissue culture and are, therefore, readily available for genetic analysis. Hopefully, genetic comparisons will be made someday to determine which factors within the virus that account for these phenomenal differences in biologic behavior.

How do feline coronaviruses exist and spread in nature? The bulk of the experimental evidence suggests that FECV strains predominate, followed by very low-virulence strains of FIPV, and finally by high-virulence strains. FECVs and low-virulence strains of FIPV appear to be spread mainly from carrier queens to their kittens within the first few weeks of life. Horizontal transmission might also occur between older carrier cats and susceptible animals of all ages. The route of virus shedding is unknown, but it probably occurs via excretions from the bowel. In utero transmission of FIPV strains has been documented in nature (McKeirnan et al., 1981; Pedersen 1983b), but the frequency or importance of in utero transmission of the infection has yet to be determined.

Where do FIPV strains come from? Are they separate entities in nature or do they arise periodically as mutations of FECV strains? Probably both situations occur to some extent. The fact that FECV-like strains can be cloned from FIPV strains, e.g. FIPV-UCD2 and high passage FIPV-TN406, indicates that the reverse situation occurs at least in the laboratory. This fluidity of virulence is not unique, however, to feline coronaviruses. A similar pattern has been seen among murine coronaviruses, which vary greatly in entero-, hepato-, and neurotropism. Strains that are very enterotropic and hepatopathic often become more and

more neurotropic as they are passaged in cell culture. The form of the disease also can vary greatly depending on the rate of inoculation and the strain of mouse that is infected (Levy et al., 1984). A similar occurrence, with more tragic overtones, was seen with the first modified live canine coronavirus vaccines recently marketed in this country. The vaccine virus caused, among other things, acute encephalitis, pancreatitis, and serositis in many dogs and had to be withdrawn quickly from the market (Martin, 1985). Not surprisingly the vaccine strain was derived from an enteric isolate.

What determines whether FIPV-infected cats become immune or develop FIP? The line between immunity and disease for most cats is distinct. Among the 21 kittens that were infected with FIPV-UCD3 or FIPV-UCD4, five developed progressive and ultimately fatal FIP, four developed transient signs of FIP and recovered, and 12 never showed any signs of illness. The course of the disease is established very quickly after initial virus dissemination, therefore. Most of the cats that died of FIP developed the effusive form; noneffusive FIP was seen in only one of five cats dying from FIP. Cats that developed the noneffusive form of FIP following experimental infection usually had a preceding and transient bout of effusive-type disease. This supports the postulate that noneffusive FIP occurs in cats that have partial protective (cellular?) immunity (Pedersen, 1983b). The granulomatous nature of noneffusive FIP suggests a partially successful attempt by the animal to wall off and contain the virus. The widespread and very active inflammatory lesions of effusive FIP, in contrast, indicate that the host is having great difficulty in containing the infection. If all of these assumptions are correct, then cats that recover from FIPV infection are those that mount a rapid and efficient cell-mediated immune response. If they fail to mount an effective defense, and clinical signs (lesions) occur in the body, the likelihood of the cat reversing the course of the disease is small.

Studies reported herein also support the theory that FIPV recovered cats are immune by a process of "infection immunity" or "premunition". Once FIPV recovered cats no longer retain reactivatable infections they seem to also lose protective immunity. In fact, they appear to become hypersensitized to a subsequent challenge-exposure. This implies that

there is a selective loss of certain types of immunity with time. It is postulated that protective immunity is largely cell mediated. This assumption is based on circumstantial evidence, but is quite compelling considering what is known about FIP and other granulomatous-type infectious diseases. It is an established fact that hypersensitizing immunity is due exclusively to humoral antibodies (Pedersen and Boyle, 1983; Weiss and Scott, 1981b). It is reasonable to assume, therefore, that as cats lose their FIPV carrier status that they also lose their cell-mediated immunity. This is not inconsistent with what is known about granulomatous diseases, where protective immunity is maintained by small foci of persistent organisms. Having lost protective immunity, the cat would possess mainly humoral immunity. Humoral immunity with concurrent loss of cell-mediated immunity would render the cat hypersensitive to challenge-exposure with a large dose of virulent FIPV.

What is the likelihood of developing a vaccine against FIP? First, it is unlikely that a killed virus vaccine will be efficacious. To be effective, the antigen must evoke a long-lasting, cell-mediated immunity. Cellular immunity can be evoked with killed antigens, but it is generally much weaker and of shorter duration than that evoked by infectious agents. The ideal vaccine would be a modified-live product derived from an FIP-inducing strain that has retained its invasiveness and will persist long enough in the body to evoke cellular immunity. FIPV-UCD3, when given oronasally, will evoke solid protective immunity in many healthy SPF kittens without inducing FIP. The problem, however, is that they become immune carriers of the virus and may develop FIP if their immunity is subsequently depressed. Another problem with using naturally attenuated strains such as FIPV-UCD3 is that they do not do anything different than what is occurring naturally in the field. The ideal vaccine strain, therefore, would be attenuated enough to still induce protective immunity but not so attenuated that it would induce sensitizing immunity. The line between the two is very fine. For instance, FIPV-UCD2 has lost all of its FIP-inducing potential and, with it, its ability to evoke protective immunity. FIPV-UCD3 has retained its immunity-evoking potential but has not lost enough of its disease-causing potential. The second problem in developing an effective FIP vaccine is that of strain variation. Cats that appear solidly immune to one or more strains of FIPV can develop FIP when inoculated with another strain. It

will be important, therefore, for vaccine manufacturers to test their products against several strains of FIPV.

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