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Epitope-Specific Antibody Responses to Virulent and Avirulent Feline Infectious Peritonitis Virus Isolates

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Feline infectious peritonitis virus (FIPV) has been isolated several times from infected cats. Some of these isolates vary markedly in their ability to cause disease. Specific-pathogen-free cats were inoculated with the avirulent FIPV-UCD-2 isolate or the extremely virulent FIPV-79-1146 isolate or both. After 1 month, cats which had received FIPV-79-1146 were either dead or showed clinical signs of FIP. All cats which received only FIPV-UCD-2 remained healthy up to 6 months after inoculation. Antibody-mediated immune enhancement of disease was not observed in cats which received FIPV-UCD-2 before inoculation with FIPV-79-1146. Monoclonal antibodies which recognized type-specific epitopes on each of the structural polypeptides of these two viruses were used in competitive-inhibition enzyme-linked immunosorbent assays to analyze the humoral immune responses of the cats. All cats produced antibodies to epitopes found on the homologous virus. In addition, cats inoculated with FIPV-79-1146 also produced antibodies which inhibited the binding of the anti-FIPV-UCD-2 E1 monoclonal antibody. One cat inoculated twice with FIPV-UCD-2 produced antibodies which inhibited the binding of the anti-FIPV-79-1146 N- and E1-specific monoclonal antibodies. Competitive enzyme-linked immunosorbent assays may prove useful in distinguishing cats which are infected with virulent FIPV isolates from cats infected with avirulent feline coronaviruses.

Feline infectious peritonitis (FIP) is an immune complex-mediated disease of domestic and exotic cats caused by a coronavirus, FIP virus (FIPV) (9, 13). There are two different forms of the disease. The first, known as effusive FIP, is characterized by peritonitis or pleuritis or both. Cats with effusive FIP often present with a chronic fever of 2 to 5 weeks duration or more, weight loss, malaise, and abdominal distension due to peritoneal fluid. The second form, known as noneffusive FIP, is characterized by microscopic or macroscopic granulomas in various parenchymatous organs. The central nervous system is often involved (9, 13). Antibodies to the virus appear to accelerate the onset of clinical signs and death in many cases (10, 15).

There are three major structural proteins in the FIPV virion: a nucleocapsid protein (N), a transmembrane envelope glycoprotein (E1), and the peplomer glycoprotein (E2). A library of monoclonal antibodies (MAbs) directed against these three structural polypeptides have been produced and characterized (6). Six FIPV isolates were divided into two distinct antigenic groups with this MAb library. One antigenic group included the more virulent FIPV isolates, including FIPV-79-1146, whereas an avirulent isolate, FIPV-UCD-2, was the sole member of the second antigenic group. In general, MAbs directed to E1 and N recognized all of the FIPV isolates (group-specific epitopes), suggesting that the antigenic sites of these structural polypetides are highly conserved. The MAbs which could discriminate between the two antigenic groups of FIPV isolates were termed type specific. All of the anti-E2 MAbs were type specific in that they recognized either one antigenic group or the other. A few anti-N and anti-E1 MAbs were found to be type specific.

To confirm that the FIPV-UCD-2 isolate was avirulent and to understand better the pathogenesis of FIP, studies were performed in vivo using the FIPV-UCD-2 isolate (12) and the extremely virulent FIPV-79-1146 isolate (11). One objective of the experiments was to determine whether preexisting antibodies to the avirulent isolate caused acceleration of clinical symptoms and death after inoculation with the virulent isolate; FIPV-naive cats which only received the virulent isolate were used for comparison. Another objective was to determine whether type-specific MAbs could distinguish virulent from avirulent FIPV infections in cats in competitive enzyme-linked immunosorbent assays (ELISAs).

MATERIALS AND METHODS

Virus isolates. FIPV-UCD-2 was a gift from N. C. Pedersen, University of California, Davis, and FIPV-79-1146 was a gift from J. Evermann, Washington State University, Pullman. Both viruses were propagated in feline whole fetus cells in RPMI 1640 supplemented with 5% fetal bovine serum, 50 U of penicillin per ml, 50 μg of streptomycin per ml, and 2 mM L-glutamine.

Virus purification. The methods of virus purification have been described previously (7). Briefly, when cytopathic effect (CPE) was 80 to 90% complete, the medium was removed from infected cultures and centrifuged at 10,000 × *g* for 10 min. Polyethylene glycol 6000 (10% [wt/vol]) was added to the virus-containing supernatant and stirred for 2 h at 4°C. The polyethylene glycol precipitate was centrifuged at 5,000 × *g* for 30 min, suspended in TEN buffer (0.05 M Tris, 0.001 M EDTA, 0.15 M NaCl [pH 6.0]), and layered onto a 30% sucrose cushion. The virus was centrifuged at 41,000 rpm in a Beckman SW41 rotor for 1 h. Pelleted virus was resuspended in TEN buffer.

Cats. Specific-pathogen-free cats (16 weeks old; Liberty Laboratories, Liberty Corner, N.J.) were maintained in individual cages in three separate rooms. All cats were found to be free of antibodies to FIPV and feline leukemia virus antigen before inoculation. On day 0, two cats were inoculated with 2×10^6 PFU of FIPV-79-1146 oronasally and held

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TABLE 1. IgG concentrations in sera during the immune response to FIPV

Cat	FIPV isolate[a]	IgG concn (mg/ml) on day:								
		0	7	14	21	28	36	43	50	57
IRN-2	UCD-2/none	4.6	5.6	6.7	5.8	5.0	4.6		8.6	8.4
IRN-3	UCD-2/none	8.0	6.7	7.6	7.1	7.6	9.6	8.2	7.6	8.6
IRV-4	UCD-2/UCD-2	5.4	4.0	5.2	4.2		5.0		5.0	5.8
IRR-4	UCD-2/UCD-2	3.4	3.9	3.6	5.6	7.6	7.6	8.6	6.0	6.0
IRN-1	1146/none	4.3	7.0	16.4	12.2	15.6				
IRI-1	1146/none	5.4	7.2	10.4	12.0	13.6	13.2			
IRK-2	UCD-2/1146	7.2	7.6	8.0	6.4	6.0	6.8	12.0	13.2	
IRS-2	UCD-2/1146	6.7	5.2	7.6	7.6	7.6	7.0	10.4	15.2	
IRN-4	None/1146	6.7	6.0	5.5	6.6	5.4	4.3	8.2	7.3	
IRR-2	None/none		4.2	3.4	4.2	4.4	3.7	5.9	5.0	5.2

[a] First inoculation/second inoculation.

in room A. Six cats were inoculated with 2×10^6 PFU of FIPV-UCD-2 oronasally and held in room B. One uninoculated contact control cat was also held in room B. One additional uninoculated cat was held in room C. On day 28, the cats inoculated with FIPV-UCD-2 were divided into three groups of two cats each. Two cats were given a second dose of 2×10^6 PFU of FIPV-UCD-2 oronasally, and two cats were moved to room A and received 2×10^6 PFU of FIPV-79-1146 oronasally; the third group was not manipulated. The contact control animal from room B was also moved to room A and given 2×10^6 PFU of FIPV-79-1146 oronasally. All cats were monitored daily for clinical symptoms. Temperatures were checked three times a week, and the cats were bled weekly.

Virus isolation. Fresh tissue from killed cats was minced and cocultivated with monolayers of feline whole fetus cells. The cells were observed daily for CPE. If no CPE was apparent in 3 to 4 days, the cultures were blind passaged on fresh feline whole fetus cell monolayers. All tissues were passed at least three times before being designated as negative. In addition to finding the characteristic CPE of feline coronaviruses in feline whole fetus cells, the presence of FIPV was confirmed in an antigen ELISA by using tissue culture supernatants. The 11G4 MAb was adsorbed to microtiter wells. Dilutions of the tissue culture supernatants and optimally diluted peroxidase-conjugated MAb 6F7 were incubated in the wells for 1 h at 37°C. After the wells were washed in TEN buffer, *o*-phenylenediamine (0.4 mg/ml; Sigma Chemical Co., St. Louis, Mo.) containing 0.01% H_2O_2 in citrate phosphate buffer (pH 5.0) was added to each well. The reaction was stopped after 15 min by the addition of 2.5 N H_2SO_4. The A_{490} was determined on a Dynatech Microelisa reader (model MR580).

Quantitation of cat IgG by single radial immunodiffusion. Assays for quantitation of cat immunoglobulin G (IgG) were performed as previously described (7) except that the slides were incubated overnight at 4°C.

MAbs to FIPV. Six previously characterized MAbs were used to analyze the humoral immune responses of cats to epitopes on the three structural polypeptides (6). Three of these recognized FIPV-UCD-2 and not FIPV-79-1146: 14G6 (anti-N), 14Cl (anti-E1), and 1F12 (anti-E2). The other three MAbs reacted with FIPV-79-1146 and not FIPV-UCD-2: 1E2 (anti-N), 11G4 (anti-E1), and 9A1 (anti-E2).

Competitive ELISAs. Microtiter plates were coated with 50 ng of purified virus diluted in TEN buffer by incubation overnight at 37°C. Free binding sites were blocked with 2% bovine serum albumin (BSA) diluted in TEN buffer by incubation for 1 h at 37°C. Competitive ELISAs were performed by adding cat serum (50 μl of a 1:10 dilution in TEN buffer–0.1% Triton X-100–0.1% BSA [TEN-TX-BSA]) to duplicate FIPV antigen-coated wells and incubating for 30 min at 37°C. Without removing the cat serum, optimally diluted MAb (50 μl in TEN-TX-BSA) was added, and incubation was continued for another 30 min at 37°C. The wells were then washed five times, and 0.1 ml of gamma-chain-specific, affinity-purified, peroxidase-conjugated goat anti-mouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted in TEN-TX-BSA was added. The plates were incubated for 30 min at 37°C and then washed five times in TEN buffer. The enzyme reaction was visualized as described above. Serum samples from day −7 and day 0 bleeds were designated negative controls, and the optical densities (ODs) of the wells were averaged. Percent competition was calculated by using the formula 100 − [(OD of test wells/OD of negative sera) × 100]. All samples were tested in duplicate on the same day to minimize possible assay variations. Duplicates were generally very close, with 83% of the pairs demonstrating 10% variation or less and 52% demonstrating 5% variation or less. Sera which demonstrated ≥35% competition were considered positive.

RESULTS

Pathogenicity of FIPV-UCD-2. All cats which received FIPV-UCD-2 either once or twice remained healthy and clinically normal for up to 6 months after the initial inoculation. None of these cats had IgG levels above 10.0 mg/ml during the time they were studied (Table 1).

Pathogenicity of FIPV-79-1146. Two specific-pathogen-free cats (IRN-1 and IRI-1) inoculated with FIPV-79-1146 developed a transient fever on day 3 postinfection (p.i.) which disappeared after 24 to 72 h, reappeared on day 17 p.i., and persisted. Both cats developed anorexia on day 7 p.i. which continued until the time of death. Cat IRN-1 developed neurological symptoms (ataxia) on day 19 p.i. and was killed on day 23 p.i. This cat was diagnosed as having noneffusive FIP at necropsy. The second cat (IRI-1) developed classical effusive FIP. Peritoneal fluid was collected on day 32 p.i., and the cat was killed on day 37 p.i. FIPV was isolated from the following organs after necropsy: the liver, spleen, mesenteric lymph nodes, and small and large intestines of both cats and the lungs, tonsils, and brain of IRN-1. The IgG levels in both cats rose above 10.0 mg/ml at day 14 p.i. and remained elevated (Table 1).

A third cat (IRN-4), which had been an uninoculated sentinel control for FIPV-UCD-2 during the first half of the experiment, was also inoculated with FIPV-79-1146. It also

developed a transient fever on day 3 p.i. which reappeared on day 14 p.i. Findings at necropsy confirmed the diagnosis of effusive FIP, and virus was isolated from the liver, spleen, mesenteric lymph nodes, lungs, tonsils, and small and large intestines. The IgG levels in this cat never exceeded 8.2 mg/ml (Table 1).

Effect of prior immunity to FIPV-UCD-2 on pathogenicity of FIPV-79-1146. Two cats (IRK-2 and IRS-2) were inoculated with FIPV-UCD-2 oronasally and 1 month later were inoculated with FIPV-79-1146 oronasally. One cat (IRS-2) developed a transient fever on day 3 after inoculation with FIPV-79-1146 which reappeared on day 14 p.i. and persisted until the cat was killed on day 25 p.i. In addition to the usual classical lesions of effusive FIP at necropsy, this cat also had severely hemorrhagic mediastinal lymph nodes. Virus was isolated from the liver, spleen, and lungs. The second cat (IRK-2) never developed a fever and died of shock while being bled on day 21 after inoculation with FIPV-79-1146. Although FIP could not be confirmed by gross findings at necropsy, virus was isolated from the lungs, tonsils, thoracic lymph nodes, and thymus. The IgG levels of both cats (IRS-2 and IRK-2) rose above 10.0 mg/ml at day 14 p.i. (Table 1). Accelerated FIP was not observed in either cat compared with cats which received only FIPV-79-1146.

Specificity of the MAbs used in the competition ELISAs. The humoral immune responses to the individual polypeptides of FIPV were analyzed by using type-specific MAbs in competitive ELISAs. The theory behind the competitive ELISAs is that if a cat produces antibodies to a given epitope on a viral structural polypeptide, these antibodies should be able to block the binding of an MAb which recognizes the same epitope. Since there were two viruses, each containing three structural proteins, six MAbs were required to conduct the competitive ELISAs. The six MAbs used are listed in Table 2 with some of their properties.

MAbs directed to N, E1, and E2 of FIPV-79-1146 recognized the corresponding epitopes on FIPV-79-1146 but did not react with FIPV-UCD-2 polypeptides; the converse was true for anti-FIPV-UCD-2 MAbs (Table 2). Four of the MAbs were of the IgG2a isotype, one was IgG1, and one was IgG2b. This permitted the use of affinity-purified gamma-chain-specific goat anti-mouse peroxidase conjugate in the competitive ELISAs. The gamma-chain-specific conjugate did not recognize cat serum at the 1:10 dilution used in the assays.

Humoral immune responses to FIPV-UCD-2 type-specific epitopes. The humoral immune responses of all cats to FIPV-UCD-2 type-specific epitopes are shown in Fig. 1. The humoral immune responses of cats inoculated with FIPV-UCD-2 are shown in Fig. 1A to D. These cats in general produced good antibody responses (>35% competition) to

TABLE 2. Virus specificity of MAbs used in competitive ELISAs

MAb	Polypeptide specificity	Isotype	Reactivity[a] with:	
			FIPV-79-1146	FIPV-UCD-2
1E2	N	IgG1	24,300	100
11G4	E1	IgG2a	24,300	100
9A1	E2	IgG2a	8,100	100
14G6	N	IgG2b	100	2,700
14C1	E1	IgG2a	100	218,700
1F12	E2	IgG2a	100	72,900

[a] Minimum reciprocal dilution of antibody required to yield an A_{490} of >0.30.

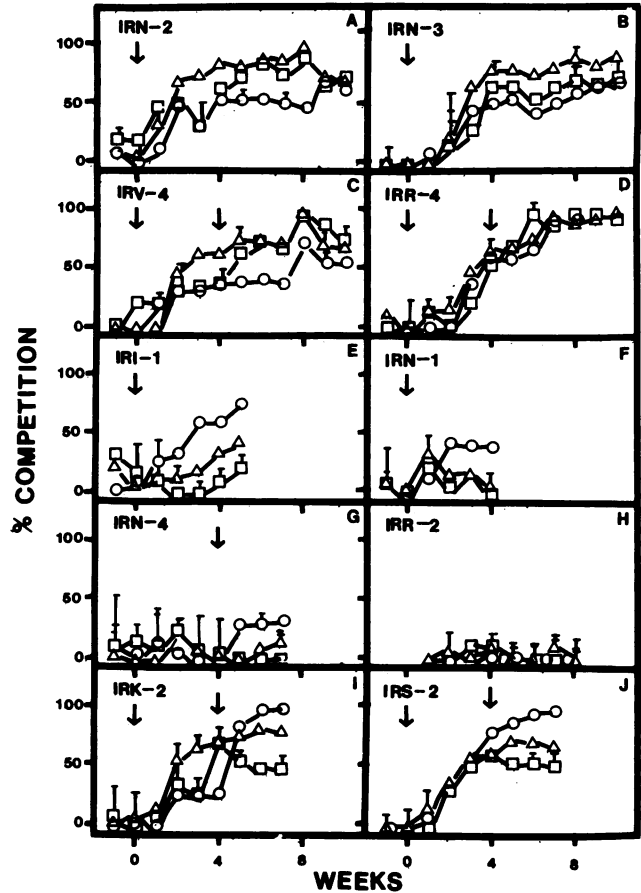

FIG. 1. Humoral immune responses (means ± standard deviations) to type-specific epitopes on FIPV-UCD-2. (A, B, C, and D) Cats inoculated with FIPV-UCD-2; (E, F, and G) cats inoculated with FIPV-79-1146; (H) uninoculated control; (I and J) cats inoculated with FIPV-UCD-2 (week 0) followed by inoculation with FIPV-79-1146 (week 4). Antibody responses were determined by competitive ELISAs. Symbols: □, MAb 14G6 (anti-N) competition; ○, 14C1 (anti-E1) competition; △, MAb 1F12 (anti-E2) competition. The arrows indicate the times of inoculation with virus. When no error bar is shown, the standard deviation was ≤3%.

the type-specific epitopes found on all three viral structural polypeptides within 2 to 3 weeks of inoculation. In three of the four cats, the response to E2 was generally higher than to E1, and the response to N was intermediate. The fourth cat (IRR-4) had comparable responses to all three polypeptides.

In contrast, the three cats inoculated with FIPV-79-1146 (IRI-1, IRN-1, and IRN-4) in general did not inhibit the binding of the type-specific MAbs to FIPV-UCD-2 E2 and N but did compete with the 14C1 MAb, which recognizes E1 of FIPV-UCD-2 (Fig. 1E to G). Serum from the uninoculated control cat did not inhibit the binding of any of the FIPV-UCD-2-specific MAbs (Fig. 1H). Cats which initially received FIPV-UCD-2 and were later inoculated with FIPV-79-1146 (IRK-2 and IRS-2) demonstrated antibody responses to FIPV-UCD-2-specific epitopes, similar to the cats which received only FIPV-UCD-2 (Fig. 1I and J).

Humoral immune responses to FIPV-79-1146 type-specific epitopes. The humoral immune responses of all cats to FIPV-UCD-2 type-specific epitopes are shown in Fig. 2. Sera from cats which received FIPV-UCD-2 (IRN-2, IRN-3, IRV-4, and IRR-4) did not inhibit the binding of the 9A1

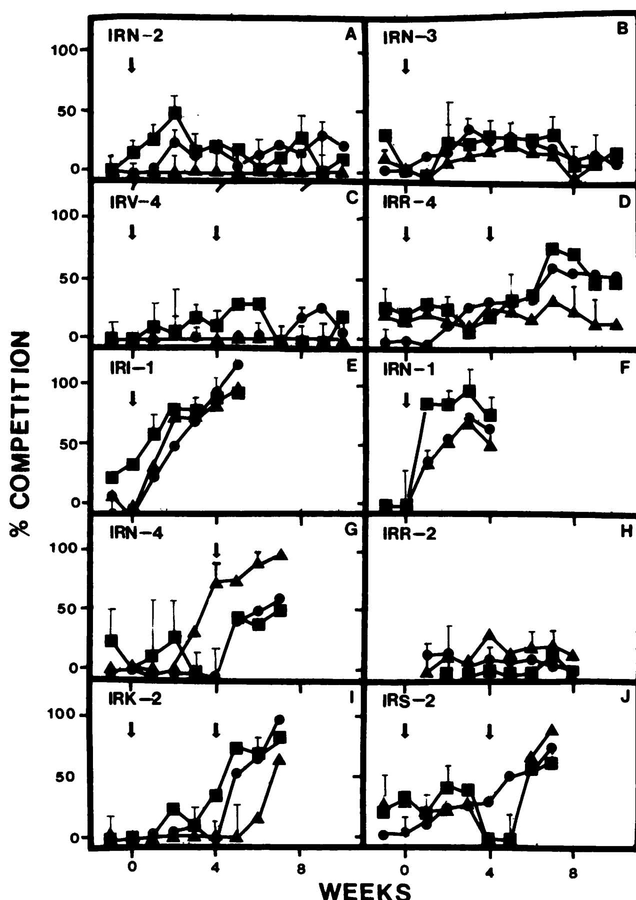

FIG. 2. Humoral immune responses (means ± standard deviations) to type-specific epitopes on FIPV-79-1146. (A, B, C, and D) Cats inoculated with FIPV-UCD-2; (E, F, and G) cats inoculated with FIPV-79-1146; (H) uninoculated control; (I and J) cats inoculated with FIPV-UCD-2 (week 0) followed by inoculation with FIPV-79-1146 (week 4). Antibody responses were determined by competitive ELISAs. Symbols: ■, MAb 1E2 (anti-N) competition; ●, MAb 11G4 (anti-E1) competition; ▲, MAb 9A1 (anti-E2) competition. The arrows indicate the times of inoculation with virus. When no error bar is shown, the standard deviation was ≤3%.

MAb, which recognizes FIPV-79-1146 E2 (Fig. 2A to D). Three of the four FIPV-UCD-2-inoculated cats had low antibody responses (usually less than 35% competition) to N and E1 of FIPV-79-1146. Serum from the fourth cat (IRR-4) inhibited the binding of the FIPV-79-1146-specific anti-E1 and anti-N MAbs starting at week 7.

Sera from cats which were inoculated with FIPV-79-1146 (IRI-1, IRN-1, and IRN-4) produced antibodies to all three polypeptides by day 7 (Fig. 2E to G). The uninoculated control cat (IRR-2) remained negative throughout the experiment (Fig. 2H). Cats which received FIPV-UCD-2 before inoculation with FIPV-79-1146 (IRK-2 and IRS-2) showed little response to FIPV-79-1146 polypeptides (usually ≤35% competition) after inoculation with FIPV-UCD-2. However, after inoculation with FIPV-79-1146, antibody responses to all three polypeptides increased (Fig. 2I and J).

DISCUSSION

The results presented here confirm previous reports that FIPV-UCD-2 is avirulent (at least for the 6-month duration of the experiment) and that FIPV-79-1146 is extremely virulent (11, 12). Cats which received only FIPV-79-1146 were killed on days 23, 27, and 37 p.i., with findings at necropsy consistent with FIP. Two cats were given FIPV-UCD-2 and responded serologically to the virus before inoculation with FIPV-79-1146. Of these, one cat died of FIP on day 25 p.i. and the other died of shock on day 21 p.i. Thus, antibody-mediated immune enhancement or accelerated FIP could not be demonstrated. This confirms the results of a previous study in which this combination of FIPV isolates was used (12).

The second objective of the study was to determine whether type-specific MAbs could distinguish virulent from avirulent FIPV in cats. To evaluate the data, a number of assumptions were made. When it was assumed that a cat should respond immunologically to an antigen within 14 days of inoculation and that the serum should inhibit the MAb by 35% or more, the results shown in Table 3 were obtained. There were 48 samples from cats inoculated with FIPV-UCD-2 from day 14 p.i. onward. These samples should have been positive only in the three FIPV-UCD-2-specific competitive ELISAs. There were 13 samples from cats inoculated with FIPV-79-1146 starting at day 14 p.i. These serum samples should have been positive only in the three FIPV-79-1146-specific competitive ELISAs. There were 30 samples from negative cats (day −7 and day 0 samples and samples from specific-pathogen-free cats) which should have been negative in all six of the competitive ELISAs.

Both anti-E2 assays were the best as far as specifically detecting only those cats which had received the homologous virus. No common epitopes have been detected on the E2 glycoproteins of FIPV-79-1146 and FIPV-UCD-2 with MAbs. Perhaps mice do not respond to common epitopes as strongly as they do to unique ones. The virus presumably does not replicate in mice as it does in cats, and this may also influence the type of antibodies generated in mice as opposed to cats. Western blots from the terminal bleeds of several cats suggested that the cat sera recognized only the homologous virus and not the heterologous virus (data not shown).

The anti-N competition assays were also fairly specific in detecting only those cats which had been inoculated with the homologous virus. Only cats which received FIPV-UCD-2 inhibited the binding of the anti-FIPV-UCD-2 N MAb (Fig. 1 and Table 3). There were seven discrepant samples in the 1E2 assay, which measured antibodies to FIPV-79-1146 N.

TABLE 3. Data obtained from type-specific competitive ELISAs

MAb	Specificity[a]	No. of positive serum samples[b] from cats inoculated with:			
		FIPV-UCD-2 (48)[c]		FIPV-79-1146 (13)	
		Observed	Expected	Observed	Expected
14C1	UCD-2 E1	39	48	6	0
1F12	UCD-2 E2	46	48	1	0
14G6	UCD-2 N	41	48	0	0
11G4	1146 E1	4	0	13	13
9A1	1146 E2	0	0	12	13
1E2	1146 N	7	0	13	13

[a] FIPV isolate and protein.

[b] The observed results assume that sera should inhibit the MAbs by 35% or more in the competitive ELISAs; the expected results assume that cats should respond humorally by day 14 and produce antibodies only to the homologous virus. There were 30 samples from control cats; the number of positive samples observed and expected in the six competitive ELISAs was zero.

[c] Number of samples.

Five of these were from cat IRR-4 during the last 5 weeks of the study.

The anti-E1 competition assays were the least specific. Although in general they did detect antibodies to the homologous virus, both assays also had a tendency to detect antibodies to the heterologous virus. The four discrepant samples in the 11G4 assay, which detected antibodies to FIPV-79-1146 E1, were all from cat IRR-4 during the last 4 weeks of the study. There were six samples from FIPV-79-1146-inoculated cats which competed with the FIPV-UCD-2 E1-specific MAb, 14C1.

There are several possible explanations for the results observed with samples from FIPV-79-1146-inoculated cats which competed with the FIPV-UCD-2-specific MAb, 14C1. First, the 14C1 MAb may not be entirely type specific. In immunoperoxidase assays of infected-cell monolayers, 14C1 weakly recognized FIPV-79-1146 at the 1/100 dilution but still reacted strongly to FIPV-UCD-2 at a 1/5,000 dilution. A 1/8,000 dilution was used in the competitive ELISA. Under the conditions used in the assay, 14C1 did not react with FIPV-79-1146 (Table 2). However, the immunoperoxidase assay results suggest that the epitope which reacts with 14C1 may exist in small quantities in the FIPV-79-1146 isolate. Since FIPV-79-1146 was probably replicating in the cats, sufficient antigenic mass might have been produced to trigger an immune response to this epitope.

Another possible explanation is that since FIPV-79-1146 was replicating in these cats, free viral antigen in the sera might also have competed with the MAb. However, free antigen could not be detected in these sera in an E1 antigen ELISA (data not shown). Circulating immune complexes or increased antibodies to common antigenic sites on the E1 glycoprotein in the sera of the FIPV-79-1146-inoculated cats might have been able to compete with the 14C1 MAb more readily. Neither of these possibilities can be ruled out. In general, the FIPV-79-1146-inoculated cats did produce higher levels of IgG (Table 1), and the FIPV-79-1146-inoculated cat with the lowest IgG levels (IRN-4) showed less competition with 14C1. However, the converse was not true. The cat with the highest IgG levels (IRN-1) did not demonstrate the highest level of competition in the 14C1 assay.

Cat IRR-4, which received two inoculations of FIPV-UCD-2, inhibited the binding of FIPV-79-1146-specific anti-N and anti-E1 MAbs from week 7 on. However, the serum did not inhibit the binding of the FIPV-79-1146 anti-E2 MAb (Fig. 2D), or an anti-pseudorabies virus MAb in an assay run under conditions exactly the same as those for the FIPV competition ELISAs (data not shown). Antibodies to common epitopes on the E1 and N polypeptides might have sterically inhibited the binding of the 1E2 and 11G4 MAbs. Antibodies to common antigenic sites on N in particular increased dramatically between weeks 6 and 7 in this cat (data not shown). Still, the question remains as to why this particular cat was so different immunologically from the other FIPV-UCD-2-inoculated cats. It may be that FIPV-UCD-2 established a persistent or systemic infection in this cat but not in the others.

The peplomer glycoprotein is thought to play an important role in the pathogenesis of other coronaviruses. For instance, passive transfer of MAbs directed against certain epitopes on E2 of mouse hepatitis virus protects mice from a lethal intracerebral virus challenge (4). Mutant mouse hepatitis virus isolates selected in the presence of neutralizing anti-E2 MAbs have been shown to have reduced neurovirulence (5, 8).

The apparent antigenic differences in the E2 glycoproteins of FIPV-79-1146 and FIPV-UCD-2 may explain the differences in the pathogenicity of these two isolates and may also explain why FIPV-UCD-2 does not sensitize cats. MAb-resistant mutants of FIPV-79-1146 might show pathogenic characteristics similar to those of the naturally occurring FIPV-UCD-2 isolate and vice versa.

The diagnosis of FIP has often proved difficult, partly because of the often generic symptoms of the disease (anorexia, fever, malaise, depression, and weight loss) and partly because there is no good diagnostic test to confirm that FIPV is actually infecting a given cat. The diagnostic tests currently used do nothing but confirm that a cat has been exposed to a coronavirus of this antigenic group at some time in its life and has mounted a humoral immune response to it (1, 9, 14). Recent vaccination often produces false-positive results in some of these diagnostic assays since the cats respond humorally to cellular or fetal bovine serum antigens present in the vaccines (2, 3). By using MAbs which recognize type-specific epitopes on the virulent FIPV isolates in competititve ELISAs, a better diagnostic test may be possible.

In summary, FIPV-UCD-2 was shown to be avirulent in cats and did not seem to sensitize cats to accelerated FIP after inoculation with FIPV-79-1146. Cats produced antibodies to all three structural polypeptides on the homologous virus and occasionally to polypeptides on the heterologous virus. The type-specific competitive ELISAs may prove useful as diagnostic tests for FIPV infection in cats.

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