JOURNAL OF VIROLOGY, Aug. 1987, p. 2607–2613
0022-538X/87/082607-07$02.00/0
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Vol. 61, No. 8

Antigenic Comparison of Feline Coronavirus Isolates: Evidence for Markedly Different Peplomer Glycoproteins

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Received 16 December 1986/Accepted 8 May 1987

The antigenic relationships among seven feline coronavirus isolates were investigated by using a panel of 26 monoclonal antibodies (MAbs). The MAbs were categorized into five immunoreactive groups which were used to delineate two antigenic types of feline coronaviruses. One antigenic type included the more virulent feline infectious peritonitis virus (FIPV) isolates (FIPV-UCD-1, FIPV-UCD-4, FIPV-TN406, FIPV-DF2, and FIPV-79-1146), whereas the second antigenic type was composed of the avirulent isolate FIPV-UCD-2. The feline enteric coronavirus isolate FECV-79-1683 shared some characteristics of both of the major antigenic groups. Epitopes on the nucleocapsid and envelope polypeptides were in general highly conserved among both antigenic types, although a few type-specific antigenic sites were discriminated. The most striking finding was the marked antigenic difference in the peplomer (E2) glycoproteins between the two antigenic types. Seven anti-E2 MAbs reacted with one antigenic type of E2, whereas seven other anti-E2 MAbs recognized a different antigenic form of E2. None of the 14 anti-E2 MAbs reacted with all of the isolates.

Feline coronaviruses are associated with at least two distinct diseases in domestic and exotic cats. Feline infectious peritonitis (FIP) is an immune complex-mediated disease caused by feline infectious peritonitis virus (FIPV) (15, 24). Feline enteric coronavirus (FECV) causes a mild or inapparent enteritis in kittens, which is distinct from FIP (18, 19). Both FIPV and FECV are similar to other coronaviruses in structure and morphology (3, 22). Each virus has three major polypeptides: a 45- to 50-kilodalton nucleocapsid protein (N); a 25- to 30-kilodalton transmembrane envelope glycoprotein (E1); and a 180- to 200-kilodalton peplomer glycoprotein (E2). These two viruses are also antigenetically related to each other and to transmissible gastroenteritis virus of swine, canine coronavirus, and human coronavirus 229E (9, 21).

The relative pathogenicity of several feline coronaviruses has been described (17, 19, 20). Their various degrees of virulence in cats suggest that these virus strains are significantly different from one another; however, to date no distinct serotypes of feline coronaviruses have been defined. As a result, antigenic differences which may correlate with degrees of virulence have not been studied.

The current study was undertaken to define antigenic relationships among different feline coronaviruses by use of a panel of monoclonal antibodies (MAbs). Some of these MAbs delineated epitopes which were highly conserved among the different viruses, particularly on the N and E1 structural components. In contrast, other MAbs were used to differentiate the E2 peplomer glycoproteins of virulent and avirulent FIPV strains.

MATERIALS AND METHODS

Virus isolates. The feline coronavirus isolates (FIPV-UCD-1, FIPV-UCD-2, FIPV-UCD-4, and FIPV-TN406) were obtained from N. C. Pedersen (University of California, Davis). FIPV-DF2 was obtained from the American Type Culture Collection (Rockville, Md.). The FIPV-79-1146 and FECV-79-1683 isolates were obtained from J. F. Evermann (Washington State University, Pullman). Isolation and characteristics of these isolates have been described previously (2, 5, 12, 13, 16, 18–20) (Table 1).

Cells. All virus isolates were grown in feline whole fetus cells, obtained from N. C. Pedersen (University of California, Davis) 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. Tissue culture fluids from virus-infected feline whole fetus cells were harvested at 36 to 48 h postinfection, when virtually all cells showed cytopathic effect. The fluids were clarified by centrifugation at 10,000 × g for 10 min and concentrated by precipitation with 10% (wt/vol) polyethylene glycol 6000 at 4°C for 2 h. The precipitate was pelleted by centrifugation at 5,000 × g for 30 min and suspended in cold TEN buffer (0.05 M Tris, 0.001 M EDTA, 0.15 M NaCl [pH 6.0]) and layered over 2 ml of 30% (wt/wt) sucrose in TEN buffer. After centrifugation at 41,000 rpm for 1 h in a Beckman SW41 rotor (Beckman Instruments, Inc., Fullerton, Calif.), the virus-containing pellet was suspended in TEN buffer.

Viral polypeptide purification. Viral polypeptides were purified by previously described procedures (23). Briefly, purified virus was disrupted with 1% Nonidet P-40 (NP-40) and layered over a 10-ml 15 to 50% (wt/wt) sucrose gradient containing 0.1% NP-40 which was on top of a 0.5-ml 65% sucrose cushion. The gradients were centrifuged at 38,500 rpm for 17 h in a Beckman SW41 rotor. Fractions (0.25 ml) were collected, diluted in TEN buffer, and adsorbed to microtiter wells by incubation overnight at 37°C. The next morning, the wells were blocked with 2% bovine serum albumin for 1 h at 37°C and probed with MAbs specific for the N, E1, or E2 polypeptide. Appropriate fractions were pooled.

MAb production. MAbs were produced by immunizing mice with either FIPV-DF2, FIPV-UCD-2, transmissible gastroenteritis virus (Miller isolate), or canine coronavirus. BALB/c mice were inoculated intraperitoneally with 100 μg of purified virus in Freund complete adjuvant, followed 2

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TABLE 1. Feline coronavirus isolates

Isolate	Source	FIP pathogenicity[a]
FECV-79-1683	Evermann, Washington State University, Pullman	−
FIPV-UCD-2	Pedersen, University of California, Davis	−
FIPV-UCD-4	Pedersen, University of California, Davis	+
FIPV-UCD-1	Pedersen, University of California, Davis	++
FIPV-DF2	American Type Culture Collection, Rockville, Md.	++
FIPV-TN406	Black, Specialized Assays, Nashville, Tenn.	++
FIPV-79-1146	Evermann, Washington State University, Pullman	++

[a] −, Has never been shown to cause FIP in experimental cats; +, sometimes causes FIP in experimental cats; ++, usually causes FIP in experimental cats.

weeks later with 50 μg of virus in Freund incomplete adjuvant. Two weeks later, the mice were boosted intravenously with 10 μg of virus in phosphate-buffered saline. Three days after the last immunization, the spleen was removed and teased to a single-cell suspension and the spleen cells were fused to SP2/0-Ag14 myeloma cells by using polyethylene glycol 4000 (6). Fused cells were selected with HAT medium (6).

Hybridoma wells were screened by an enzyme-linked immunosorbent assay (ELISA). Initially, cultures were chosen which reacted with the immunizing virus and not uninfected cell lysate or unrelated viruses (feline leukemia virus or feline panleukopenia virus). In later experiments, MAbs were chosen which specifically recognized one feline coronavirus isolate (e.g., FIPV-UCD-2) but not another (e.g., FIPV-DF2).

After being cloned several times by limiting dilution, hybridoma cells were injected into pristane-primed BALB/c mice for ascites fluid production. Antibody from ascites fluid was purified by ammonium sulfate precipitation and conjugated to horseradish peroxidase by the periodate method (26). A more detailed description of these procedures has already been published (6).

Determination of immunoglobulin isotype. Isotype was determined by an ELISA. Goat anti-mouse Fab (Cappel Worthington, Malvern, Pa.) was adsorbed to microtiter wells. Hybridoma culture supernatants were added, incubated for 30 min at 37°C, and aspirated. The wells were washed four times in TEN buffer. Rabbit antisera specific for mouse immunoglobulin M (IgM), IgG1, IgG2a, and IgG2b (Litton Bionetics, Kensington, Md.) were added and incubated for 30 min at 37°C. The plates were washed four times with TEN buffer, and horseradish peroxidase-conjugated goat anti-rabbit IgG (Cappel Worthington) was added and incubated for 30 min at 37°C. The plates were then washed four times in TEN buffer, and the chromagen substrate (50 μl of 0.4 mg *o*-phenylenediamine per ml [Sigma Chemical Co., St. Louis, Mo.]–0.01% H_2O_2) in citrate phosphate buffer (pH 5.0) was added. The plates were incubated for 15 min at room temperature, and the reaction was stopped by the addition of 50 μl of 2.5 N H_2SO_4. The A_{490} was measured on a Dynatech MR580 ELISA reader (Dynatech Laboratories, Inc., Alexandria, Va.).

Plaque reduction assay. Twofold serial dilutions of mouse ascites fluid starting at 1:25 were prepared in RPMI 1640 with 5% fetal bovine serum. A predetermined amount of virus that contained approximately 50 PFU was added to each dilution of MAb and incubated for 1 h at room temperature. A 0.2-ml sample of each sample was inoculated onto monolayers of feline whole fetus cells grown in six-well tissue culture plates (Becton Dickinson Labware, Oxnard, Calif.). The plates were incubated for 1 h at 4°C with frequent rocking. The inoculum was aspirated, the monolayers were washed with RPMI 1640, and 2 ml of 1% Noble agar in RPMI 1640 was added to each well. The plates were incubated for 2 to 3 days at 37°C. The number of plaques was determined after the monolayers were stained with 0.25% crystal violet in 20% methanol.

Determination of polypeptide specificity. In most instances, polypeptide specificity was determined by immunoblotting as described previously (6). However, in some cases the MAb did not recognize sodium dodecyl sulfate-disrupted proteins. For these MAbs, the polypeptide specificity was determined by an ELISA by using NP-40-disrupted, sucrose gradient-purified viral polypeptides. Pooled fractions from NP-40-disrupted, sucrose gradient-purified virus were dried in microtiter plates overnight at 37°C. After blocking for 1 h with 2% bovine serum albumin in TEN buffer, dilutions (1:100 or 1:1,000) of mouse ascites fluids were incubated in the wells for 1 h at 37°C. The wells were washed in TEN buffer and peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel Worthington) was added to the wells. After another hour of incubation at 37°C, the wells were washed four times in TEN buffer and the chromagen substrate in citrate phosphate buffer (pH 5.0) was added. The plates were incubated for 15 min at room temperature, and the reaction was stopped by the addition of 50 μl of 2.5 N H_2SO_4. The A_{490} was measured on a Dynatech MR580 ELISA reader.

Competitive inhibition ELISA. Peroxidase-conjugated MAbs were titrated against 50 ng of either FIPV-DF2 or FIPV-UCD-2 in microtiter plates. This amount of antigen was shown to be limiting in preliminary experiments. Dilutions of the conjugated MAb which gave absorbance values of approximately 1.00 in the ELISA were chosen for the competitive assays. Nonspecific binding of the labeled antibodies was assayed in wells coated with feline leukemia virus and was insignificant in all cases. To perform the competition assays, nonconjugated ascites fluid was first titrated by using serial twofold dilutions (50 μl per well) starting at 1:100 in wells containing 50 ng of either FIPV-DF2 or FIPV-UCD-2. After incubation for 30 min at 37°C, the predetermined dilution of peroxidase-conjugated antibody (50 μl) was added to the wells without removing the ascites fluid dilution. After 1 h at 37°C, the wells were washed five times and chromagen substrate was allowed to react as described above. The percent competition was determined by the formula [1 − (OD_{490} of peroxidase-conjugated MAb in the presence of competing MAb/OD_{490} of peroxidase-conjugated MAb in the absence of competing MAb)] × 100, where OD_{490} is optical density at 490 nm. In all assays, the homologous MAb was included as a positive control. Antibodies were considered to inhibit completely if they competed 80% or greater and were considered noncompetitors if they inhibited 40% or less. Antibodies showing 41 to 79% inhibition were considered partial competitors.

ELISA reactivity of MAbs to virus isolates. Purified virus was normalized for nucleocapsid antigen by use of a nucleocapsid-specific antigen ELISA (6). Twofold serial dilutions of virus starting at 1 μg in TEN buffer were

TABLE 2. ELISA reactivity of the anti-coronavirus MAbs to feline coronavirus isolates

MAb	Immunizing virus	ELISA reactivity to the following feline coronavirus isolates:						
		FIPV DF2	FIPV TN406	FIPV 79-1146	FIPV UCD-1	FIPV UCD-2	FIPV UCD-4	FECV 79-1683
Group I								
52A4	DF2	++[a]	++	++	++	++	++	++
16G7	TGEV	++	++	+[b]	+	++	++	++
3F4	TGEV	++	++	++	+	++	++	++
52D5	DF2	++	++	++	+	+	+	++
13G1	DF2	++	++	++	++	++	++	++
6F7	CCV	++	++	++	+	++	++	++
10H11	TGEV	++	++	++	++	++	++	++
11G4	DF2	++	++	++	++	+	++	+
Group II								
17F3	TGEV	++	+	++	++	–[c]	++	++
1H1	CCV	++	++	++	++	–	++	++
4E1	CCV	++	++	+	+	–	++	+
17A6	TGEV	++	++	+	+	–	++	++
9A6	DF2	++	++	++	++	–	++	++
9D11	DF2	++	++	++	++	–	++	++
Group III								
1E2	DF2	++	++	++	++	–	++	–
9A1	DF2	++	++	++	++	–	++	–
Group IV								
4G1	UCD-2	–	–	–	–	++	–	–
5F8	UCD-2	–	–	–	–	++	–	–
1F12	UCD-2	–	–	–	–	++	–	–
5B2	UCD-2	–	–	–	–	++	–	–
3E4	UCD-2	–	–	–	–	++	–	–
2E7	UCD-2	–	–	–	–	++	–	–
2D10	UCD-2	–	–	–	–	++	–	–
14G6	UCD-2	–	–	–	–	+	–	–
14C1	UCD-2	–	–	–	–	++	–	–
Group V								
2D8	UCD-2	–	–	–	–	++	–	+

[a] ++, A_{490} of ≥ 0.80.
[b] +, $A_{490} = 0.21$ to 0.79.
[c] –, A_{490} of ≤ 0.20.

adsorbed to microtiter wells overnight at 37°C. The wells were next blocked with 2% bovine serum albumin in TEN buffer. Ammonium sulfate-precipitated (43%) antibody was added at a constant concentration of 1 μg per well, and the plates were incubated for 1 h at 37°C. The wells were washed in TEN buffer, peroxidase-conjugated goat anti-mouse IgG was added, and the plates were incubated for an additional hour at 37°C. After the wells were washed in TEN buffer, chromagen substrate was added and the assay was completed as described above.

RESULTS

ELISA reactivity of the MAbs with feline coronavirus isolates. Approximately 75 MAbs were produced initially from mice inoculated with either FIPV-DF2, transmissible gastroenteritis virus of swine (Miller strain), or canine coronavirus. Some of these were specific for transmissible gastroenteritis virus of swine or canine coronavirus, or both, and did not recognize feline coronaviruses. These MAbs were not used in this study. Preliminary results with the existing anti-feline coronavirus MAbs suggested that the peplomer of FIPV-UCD-2 was antigenically different from the E2 glycoprotein of the other feline coronavirus isolates. Later, fusions were designed to produce MAbs which could distinguish specific isolates (e.g., FIPV-UCD-2) from the other isolates. The final panel of MAbs used in this study could be divided into five groups on the basis of the reactivity of each of the MAbs to 100 ng of the various feline coronavirus isolates (Table 2). Eight MAbs reacted to all seven virus preparations (group I). Six of the MAbs reacted with all the viruses except FIPV-UCD-2 (group II). The two group III MAbs did not react with FIPV-UCD-2 or with FECV-79-1683, the enteric coronavirus isolate, but did react with the other five viruses. All nine of the group IV MAbs reacted specifically with the FIPV-UCD-2 isolate. The single group V MAb reacted with both FIPV-UCD-2 and FECV 79-1683, but not with the remaining viruses.

To determine whether the differences observed were merely quantitative and not qualitative, virus concentration was increased 10-fold and the ELISA experiment was repeated. Representative titration curves are depicted in Fig. 1. The top row shows the reactivities of three of the isolates, FIPV-TN406, FIPV-UCD-4, and FIPV-UCD-2, with one of the anti-N MAbs, 52A4. Similar titration curves were observed with all three isolates with this antibody. In a similar fashion, the second row shows the reactivities of the same three isolates with one of the anti-E1 MAbs, 6F7. Again, similar titration curves were observed for all three isolates. In contrast, when the isolates were tested against an anti-

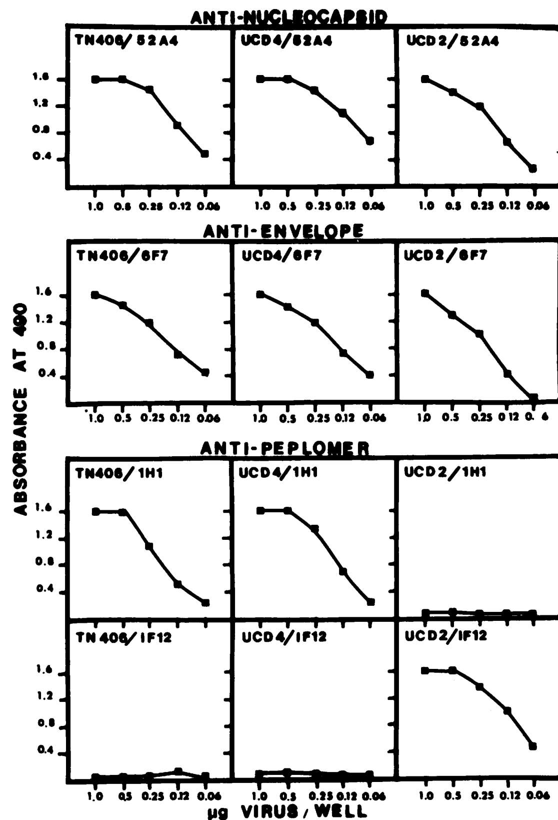

FIG. 1. ELISA reactivity of representative MAbs to three feline coronavirus isolates. Serial twofold dilutions of FIPV-TN406, FIPV-UCD-4, or FIPV-UCD-2 were adsorbed to microtiter plates and probed with ammonium sulfate-precipitated MAbs at a constant concentration of 1 μg per well. 52A4 = anti-N; 6F7 = anti-E1; 1H1 = anti-E2; 1F12 = anti-E2.

FIPV-DF2 E2 MAb (1H1), only FIPV-TN406 and FIPV-UCD-4 reacted and FIPV-UCD-2 did not. When one of the FIPV-UCD-2-specific anti-E2 MAbs was used (1F12), only FIPV-UCD-2 reacted and FIPV-TN406 and FIPV-UCD-4 did not.

Group III MAbs reacted only with the FIPV strains that can cause FIP in cats, and therefore these MAbs can be used to identify the virulent isolates. In contrast, group IV MAbs recognize only the avirulent FIPV-UCD-2 strain. The combined use of MAbs from the various groups can also be used to differentiate the viruses associated with FIP from the virus associated with feline enteritis, FECV 79-1683.

Other characteristics of the MAbs. To further localize the various feline coronavirus antigenic differences, the polypeptide specificity of each of the MAbs was determined by its reactivity to each of the three major structural components of the FIPV virion either by immunoblotting of sodium dodecyl sulfate-polyacrylamide gels or by ELISA. For the ELISA, the three structural components of FIPV were separated by sucrose gradient centrifugation of detergent-disrupted FIPV virions (Fig. 2). Dilution and analysis of the gradient fractions with MAbs whose polypeptide specificity had previously been determined by immunoblotting demonstrated three separate peaks of immunoreactivity. Fractions 1 to 6 contained N reactivity. The peplomer glycoprotein E2 was localized in fractions 16 to 24, whereas the envelope glycoprotein E1 sedimented in fractions 27 to 34. Each of the

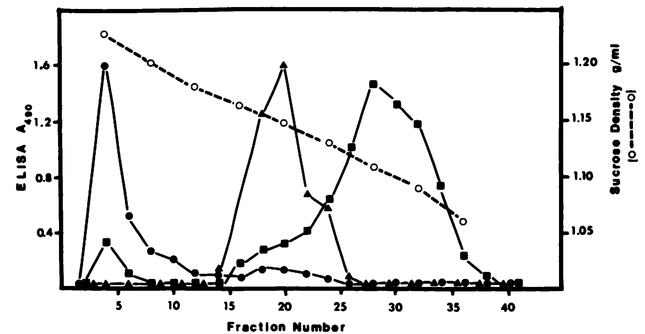

FIG. 2. Sucrose gradient distribution of feline coronavirus structural polypeptides after disruption of the viral envelope with 1% NP-40. A 1-ml amount of virus was sedimented into a 15 to 50% linear sucrose gradient in TEN buffer with a cushion of 65% sucrose. Fractions were collected and probed with MAbs specific for N (●), E1 (■), or E2 (▲). ELISA reactivity for each of the three MAbs is plotted versus fraction number.

panel of MAbs was reacted with the three separate FIPV polypeptides to determine its specificity.

The polypeptide specificity of each of the MAbs and its respective isotype are listed in Table 3. Eight of the MAbs recognized the N polypeptide, four reacted with E1, and fourteen were specific for E2. Five of the eight anti-N MAbs demonstrated group I reactivity; the remaining three had either group III, group IV, or group V reactivity. Three of the four anti-E1 MAbs showed group I reactivity, with the fourth reacting only to FIPV-UCD-2 (group IV). A total of 14 anti-E2 MAbs were defined. Six of the anti-E2 MAbs did not

TABLE 3. Properties of the anti-coronavirus MAbs

MAb	Polypeptide specificity		Group	Isotype	PRN titer[a]	
	Blot	ELISA			FIPV-DF2	FIPV-UCD-2
52A4	N		I	G1	<25	<25
16G7	N		I	M	<25	<25
3F4	N		I	G2a	<25	<25
52D5	N		I	G2a	<25	<25
13G1	N		I	G1	<25	<25
1E2	N		III	G1	<25	<25
2D8		N	V	G1	<25	<25
14G6		N	IV	G2b	<25	<25
6F7	E1		I	G1	<25	<25
10H11	E1		I	G1	<25	<25
11G4	E1		I	G2a	<25	<25
14C1		E1	IV	G2a	<25	<25
17F3	E2		II	G1	<25	<25
1H1	E2		II	G1	160,000	<25
4E1	E2		II	G2b	<25	<25
17A6	E2		II	G1	<25	<25
9D11	E2		II	G2a	<25	<25
9A6	E2		II	M	160,000	<25
9A1	E2		III	G2a	<25	<25
4G1		E2	IV	G1	<25	100
5F8	E2		IV	G2a	<25	<25
1F12	E2		IV	G2a	<25	<25
5B2		E2	IV	G2a	<25	<25
3E4		E2	IV	G2a	<25	<25
2E7		E2	IV	G1	<25	<25
2D10		E2	IV	G2a	<25	<25

[a] PRN, Plaque reduction neutralization.

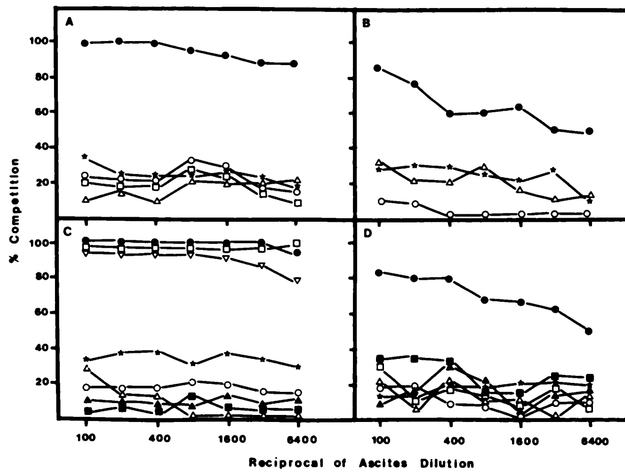

FIG. 3. Representative competitive-inhibition curves. Serial twofold dilutions of MAb ascites fluid were incubated on FIPV antigen-coated microtiter plates for 30 min. Without removal of the competitor, optimally diluted peroxidase-conjugated MAb was added and allowed to incubate for 60 min. Symbols: (A) 13G1-conjugated MAb: ●, 13G1; ★, 52A4; △, 16G7; ○, 3F4; □, 52D5 ascites fluid. (B) 6F7-conjugated MAb: ●, 6F7; ★, 10H11; △, 11G4; ○, 14C1 ascites fluid. (C) 9D11-conjugated MAb: ●, 9D11; ▲, 17F3; ★, 1H1; △, 4E1; ○, 17A6; □, 10G11; ■, 9A1; ▽, 9A6 ascites fluid. (D) 5F8-conjugated MAb: ●, 5F8; ★, 1F12; △, 4G1; ○, 5B2; □, 3E4; ▲, 2E7; ■, 2D10 ascites fluid.

react with FIPV-UCD-2 but did react with the other viruses. Another seven anti-E2 MAbs were reactive only with FIPV-UCD-2. The remaining anti-E2 MAb reacted with all virulent FIPV strains but not with FIPV-UCD-2 or with FECV 79-1683.

Most of the MAbs were either of the IgG1 isotype (12 of 27) or of the IgG2a isotype (11 of 27). There were two IgM and two IgG2b MAbs. The various MAbs were also tested for their abilities to neutralize either FIPV-DF2 or FIPV-UCD-2 in a plaque reduction neutralization assay (Table 3). None of the anti-N or anti-E1 MAbs showed a 50% reduction in plaques at a 1:25 dilution. Two anti-E2 MAbs had significant neutralization titers to FIPV-DF2 but did not neutralize FIPV-UCD-2. Only MAb 4G1 had a measurable neutralization titer to FIPV-UCD-2.

Competitive-inhibition ELISA. The reactivities of the various MAbs to the different feline coronavirus isolates (Table 2) and to the three virion polypeptides (Table 3) suggested that different epitopes were being recognized by the different MAbs on each of the virion structural components. The epitopes defined by the MAbs were further analyzed by competitive-inhibition ELISA. Each of the MAbs was tested for its ability to compete for the binding of peroxidase-conjugated MAbs to FIPV. The anti-E2 MAb had to be analyzed in two separate groups since there was no cross-reactivity between the FIPV-DF2-specific and the FIPV-UCD-2-specific MAbs. Representative competition titration curves are shown in Fig. 3, and the data are summarized in Table 4.

Many of the original 75 MAbs recognized similar epitopes in that they mutually competed with each other in competitive-inhibition assays. Significant competition was typically observed with competitor ascites fluid dilutions between 1:100 and 1:6,400. Only the MAbs which appeared to recognize unique or distinguishable epitopes were used in this study.

Nine MAbs were generated from the first FIPV-UCD-2-specific fusion. Eight of these recognized the peplomer glycoprotein, and two of these reciprocally inhibited the binding of the other MAb. The remaining seven MAbs appeared to recognize unique epitopes. It required additional anti-FIPV-UCD-2 fusions and screening of several thousand potential FIPV-UCD-2-specific MAbs to generate the anti-N (14G6) and anti-E1 (14C1) MAbs.

Each of the MAbs listed in Table 4 had its own pattern of reactivity. Since we had previously eliminated from consideration the MAbs which mutually inhibited each other, no two MAbs reciprocally competed greater than 80%. Some of the peroxidase-conjugated MAbs were more easily competed than others. This effect was most likely caused by an alteration in the binding affinity of the MAbs due to the peroxidase conjugation.

Eight different reactivity patterns were obtained with the anti-N MAbs, and four different patterns were seen with the anti-E1 MAbs. Several of the anti-N MAbs showed mutual partial competition, suggesting spatially close, yet different, epitopes. The one-way competition observed with the anti-E1 MAb 11G4 peroxidase conjugate was suggestive of a much-reduced binding affinity for this MAb.

The first group of anti-E2 MAbs in Table 4 was specific for the FIPV-DF2 type of virus. Two of the MAbs, 9A6 and 9D11, reciprocally competed. However, the neutralization data (Table 3) suggested that these two MAbs must be different; 9A6 had a significant neutralization titer, but 9D11 did not neutralize. None of the other five MAbs in this group reciprocally competed.

The second group of anti-E2 MAbs showed seven different reactivity patterns. Six of the seven MAbs in this group reciprocally inhibited only the isologous MAb, indicating at least six unique epitopes. The binding of 4G1 MAb was readily blocked by several of the other MAbs in this group. However, MAb 4G1 was the only MAb which had a neutralizing titer to FIPV-UCD-2 (Table 3), suggesting that it is different from the other six MAbs. The fact that there might be as many as seven distinct epitopes on the E2 of FIPV-UCD-2, none of which are found on the virulent FIPV strains, reinforces the extreme antigenic diversity associated with the peplomer glycoproteins of feline coronaviruses.

DISCUSSION

The data presented in this report represent the first study of feline coronavirus antigens using the exquisite specificity of MAbs. The MAbs were categorized into five immunoreactive groups, which were used to delineate two major antigenic types of feline coronaviruses. Distinct antigenic sites were determined on each of the three virion polypeptides with the panel of 26 MAbs used.

Competitive-inhibition ELISAs suggested that there are at least eight distinct epitopes on the N polypeptide of feline coronaviruses (Table 4). Five of these epitopes are common to all of the isolates. One (1E2) occurs only in the more virulent FIPV isolates, and another (14G6) is found only on the avirulent FIPV-UCD-2 isolate. The 2D8 MAb recognizes only FIPV-UCD-2 and FECV-79-1683 (Table 2). Although the nucleocapsid or core proteins of most viruses show a conservation of epitopes, type-specific epitopes can be identified by using MAbs.

Anti-E1 MAbs did not demonstrate as much diversity as anti-N MAbs; nevertheless, at least four distinct antigenic sites could be distinguished. Three of the four epitopes were found to various degrees on all the feline coronavirus iso-

TABLE 4. Competitive-inhibition ELISAs between MAbs to feline coronaviruses

	Result with competing MAb:																									
Peroxidase-conjugated MAb	52A4	16G7	3F4	52D5	13G1	1E2	2D8	14G6	6F7	10H11	11G4	14C1	17F3	1H1	4E1	17A6	9A1	9A6	9D11	4G1	5F8	1F12	5B2	3E4	2E7	2D10
Anti-N																										
52A4	+[a]	−	−	−	+/−	+/−	−	+/−																		
16G7	−	+	−	−	−	−	+/−	+/−																		
3F4	−	−	+	−	−	−	−	−																		
52D5	−	−	−	+	−	+/−	+	+																		
13G1	−	−	−	−	+	−	−	−																		
1E2	+/−	−	−	−	+/−	+		−																		
2D8	+	+	+	−	+	−	+	−																		
14G6	+/−	−	+/−	−	+/−	+/−	+	+																		
Anti-E1																										
6F7									+	−	−	−														
10H11									+/−	+	−	+/−														
11G4									+	+	+	−														
14C1									−	−	−	+														
Anti-E2																										
Type 1																										
17F3													+	−	+/−	−	−	−	−							
1H1													−	+	−	−	−	+/−	−							
4E1													−	−	+	−	−	−	−							
17A6													+/−	−	−	+	−	+/−	−							
9A1													−	−	−	+	+	−	−							
9A6													−	−	−	−	−	+	+							
9D11													−	−	−	−	−	+	+							
Type 2																										
4G1																				+	+	+	−	+	+/−	+
5F8																				−	+	−	−	−	−	−
1F12																				−	−	+	−	−	−	−
5B2																				−	−	−	+	−	−	−
3E4																				−	−	−	−	+	−	−
2E7																				−	−	−	−	−	+	−
2D10																				−	−	−	−	−	−	+

[a] +, >80% inhibition; +/−, 40 to 79% inhibition; −, <40% inhibition.

lates tested. One anti-E1 MAb (11G4) reacted best with the more virulent FIPV isolates. Another MAb (14C1) recognized only the avirulent FIPV-UCD-2 isolate. The other two anti-E1 MAb (6F7 and 10H11) seemed to react equally well to all of the isolates (Table 2). The reduced diversity demonstrated by anti-E1 MAb may be related to the transmembrane location of this virion component. In other coronaviruses, approximately 5 to 10% of the E1 is accessible to the exterior of the virion (1).

Since broadly reacting anti-E1 and anti-N MAbs were readily obtained, it was surprising to find such striking antigenic differences in the E2 glycoproteins. Seven anti-E2 MAbs reacted with one antigenic type of E2, and seven other anti-E2 MAbs recognized a different antigenic form of E2. None of the 14 anti-E2 MAbs reacted with all isolates (Table 2).

There appear to be at least seven epitopes on the E2 glycoprotein of the virulent FIPV isolates typified by FIPV-DF2 (Table 4). Two of these sites are defined by the neutralizing MAbs 1H1 and 9A6. These two neutralization epitopes may be slightly overlapping, since 9A6 partially inhibited the binding of 1H1, but not the reciprocal. Four of the remaining anti-E2 MAbs in this immunoreactive group, although nonneutralizing under the assay conditions used, represent distinct epitopes on this type of E2. The MAb 9A1 could be used to delineate FECV-79-1683 since this virus appears to have lost this epitope (Table 2).

There are at least seven distinct epitopes on the FIPV-UCD-2 type of peplomer (Table 4). One of these epitopes is defined by a weakly neutralizing antibody (4G1). The other six MAbs define nonoverlapping FIPV-UCD-2-specific epitopes.

Hybridomas of our earlier fusions were screened only for their ability to react with coronaviruses by an ELISA. There was no intentional selection for particular epitopes on the E2 glycoprotein of any coronavirus. Of the seven anti-E2 MAbs from earlier fusions, none reacted with FIPV-UCD-2. Later hybridomas were selected on the basis of reactivity only with FIPV-UCD-2 and not FIPV-DF2. Many MAbs which reacted with both FIPV-DF2 and FIPV-UCD-2 were discarded. Although some of these discarded hybridomas might have reacted with shared epitopes on the E2 glycoproteins of FIPV-DF2 and FIPV-UCD-2, it seems significant that only 3 of the 10 MAbs selected for specific reactivity to FIPV-UCD-2 reacted with either E1 or N. Fusions performed to obtain additional FIPV-UCD-2-specific MAbs have generated only 1 anti-N and 27 anti-E2 MAbs. Competitive ELISAs have not yet been done to determine whether these new MAbs react with epitopes already recognized by the existing FIPV-UCD-2-specific MAbs or whether they are specific for additional unique sites on the virion (data not shown). These data strengthen the idea of strong conservation of antigenic determinants, especially on the E1 and N polypeptides of feline coronaviruses and the extreme degree of divergence on the E2 glycoprotein.

These data suggest that there are at least two antigenic groups of FIPV. One antigenic group of FIPV is typified by FIPV-DF2 and includes FIPV-UCD-1, FIPV-UCD-4, FIPV-TN406, and FIPV-79-1146. These isolates have been shown to be usually quite virulent in cats (19, 20). The second

antigenic group consists of FIPV-UCD-2, which has been shown to be avirulent in cats (20). FECV-79-1683 appears to share some epitopes with the virulent FIPV strains as well as with the avirulent FIPV-UCD-2 strain (Table 2). FECV-79-1683 may be a mutant strain of a more virulent isolate which has lost its ability to cause FIP but has retained many of the antigenic characteristics of the more virulent strains. Mutations are known to occur readily in coronaviruses (4, 7). Alternatively, FECV-79-1683 might be the result of a recombination event between FIPV-UCD-2 and one of the more virulent strains. Genetic recombination has been demonstrated in vitro for mouse hepatitis virus, another coronavirus (10, 11).

Antigenically different peplomer glycoproteins may play a role in the pathogenesis of FIP, which is thought to be an immune complex-mediated disease. Cats do not die of FIP unless and until they mount a humoral immune response to the virus (14, 17). In fact, FIP pathogenesis has been compared to the antibody-mediated immune enhancement phenomenon observed in dengue shock syndrome (25). In dengue shock syndrome, nonneutralizing or weakly neutralizing antibodies to one serotype of dengue virus enhanced the replication of a different dengue virus serotype perhaps by facilitating opsonization into macrophages where the virus replicates (8). FIPV has been shown to replicate in macrophages (13), but until now the existence of distinct serotypes of FIPV has been merely surmised. Perhaps the FIPV-DF2 and FIPV-UCD-2 antigenic groups of FIPV demonstrated here by differential immunoreactivity to MAbs, especially on the E2 glycoprotein, represent two serotypes of FIPV which allow this immune enhancement to occur.

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