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Antigenic analysis of feline coronaviruses with monoclonal antibodies (MAbs): Preparation of MAbs which discriminate between FIPV strain 79-1146 and FECV strain 79-1683

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ABSTRACT

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We prepared 31 monoclonal antibodies (MAbs) against either FIPV strain 79-1146 or FECV strain 79-1683, and tested them for reactivity with various coronaviruses by indirect fluorescent antibody assay (IFA).

Sixteen MAbs which reacted with all of the 11 strains of feline coronaviruses, also reacted with canine coronavirus (CCV) and transmissible gastroenteritis virus (TGEV). In many of them, the polypeptide specificity was the recognition of transmembrane (E1) protein of the virus. We succeeded in obtaining MAbs which did not react with eight strains of FIPV Type I viruses (showing cell-associated growth) but reacted with FIPV Type II (79-1146, KU-1) and/or FECV Type II (79-1683) viruses (showing non-cell associated growth). These MAbs also reacted with CCV or TGEV. These MAbs recognized peplomer (E2) glycoprotein, and many antigenic differences were found in this E2 protein.

These results suggest that FIPV Type II and FECV Type II viruses are antigenically closer to TGEV or CCV than to FIPV Type I viruses. Furthermore, the MAb prepared in this study has enabled discrimination between FIPV strain 79-1146 and FECV strain 79-1683, which was thought to be impossible by the previous serological method.

INTRODUCTION

For serological diagnosis of feline infectious peritonitis virus (FIPV) infection, detection of antibody by indirect fluorescent antibody assay (IFA) is popular (Pedersen, 1976b; Horzinek and Osterhaus, 1979; Scott, 1979). On the other hand, feline enteric coronavirus (FECV) which antigenically cross-reacts with FIPV, and causes only mild enteritis without inducing FIP, may

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be present (McKeirnan et al., 1981; Pedersen et al., 1981a; Pedersen et al., 1981b; Pedersen et al., 1984). Thus, the serological diagnosis of FIP and the mechanisms of its onset become more complex. Pedersen et al. (1984a) classified the feline coronaviruses in terms of the disease types. They divided FIPV into Types I and II according to the presence or absence of the induction of FIP, ability of the viruses to proliferate in cell cultures, and the antigenic relationship with porcine and canine coronaviruses. FECV has been divided into Types I and II in the same way. Types I and II of FIPV in this classification can be serologically discriminated by the neutralization test. FIPV Type I and FECV Type II can also be distinguished by the neutralization test. Cultivation of FECV Type I in cells is not possible at present, and its serological position remains unclear. On the other hand, even the neutralization test cannot discriminate between FIPV Type II and FECV Type II. It goes without saying that, since all feline coronaviruses show cross-reaction in IFA, it is impossible to discern the types of virus infection. There are many healthy but FIPV antibody-positive cats living outdoors. As long as FIPV Type II and FECV Type II cannot be distinguished serologically, the clinical diagnostic significance of antibody detection in such cats is low.

In this study, we attempted to distinguish between the 79-1146 strain classified as FIPV Type II and the 79-1683 strain classified as FECV Type II, by means of monoclonal antibodies (MAbs). At the same time, we examined feline coronaviruses for antigenic differences by using the MAbs. We also investigated the antigenic relationship between feline coronaviruses and canine and porcine coronaviruses.

MATERIALS AND METHODS

Cell cultures. Feline whole fetus cells (fcwf-4), Crandell feline kidney cells (CrFK) and Swine kidney cells (CPK) were grown in Eagle's minimum essential medium (MEM) containing 20% Leibovitz L-15 Medium (L-15) 10% fetal calf serum, 100 units ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin. The maintenance medium was MEM containing 20% L-15 and antibiotics as above. The cells were maintained in a humidified 5% CO_2 incubator at 37°C.

Viruses. The coronavirus isolates used in this study and their sources are shown in Table 1. Among these virus strains, the authors isolated the strain KU-1 of FIPV from the liver cells of a kitten with the effusive form of FIP, and the strain KU-2 of FIPV from the peritoneal cells of an adult cat, also with effusive FIP. Among the FIPV strains used in the study, strains UCD-1, NW-1, UCD-2, UCD-3, UCD-4, Black, Yayoi and KU-2 show cell-associated growth, and are therefore regarded as Type I virus strains in the classification of Pedersen et al. (1984a). Moreover, since strain KU-1, like strain 79-1146,

TABLE 1

Source or coronavirus isolates

Virus strain		Source	Reference
FIPV	79-1146	M.C. Horzinek, State University Utrecht, The Netherlands	Pedersen et al., 1984b McKeirnan et al., 1981
	UCD-1	N.C. Pedersen, University of California, Davis	Pedersen et al., 1981a Pedersen, 1976a
	NW-1	J.K. Yamamoto, University of California, Davis	Pedersen et al., 1981a Pedersen and Black, 1983
	UCD-2	J.K. Yamamoto, University of California, Davis	Pedersen and Floyd, 1985
	UCD-3	J.K. Yamamoto, University of California, Davis	Pedersen and Floyd, 1985
	UCD-4	J.K. Yamamoto, University of California, Davis	Pedersen and Floyd, 1985
	Black	J.K. Yamamoto, University of California, Davis	Black, 1982 Pedersen and Black, 1983
	Yayoi	M. Hirano, University of Iwate, Japan	Hayashi et al., 1981
	KU-1	Author et al.	
	KU-2	Author et al.	
FECV	79-1683	A.J. McKeirnan, Washington State University, Pullman	McKeirnan et al., 1981 Pedersen et al., 1984b
TGEV	TO-163	National Institute of Animal Health of Japan	Furuuchi et al., 1975
	SH	National Institute of Animal Health of Japan	Harada et al., 1967
CCV	1-71	E. Takahashi, University of Tokyo, Japan	Binn et al., 1975

grows well even in CrFK cells in a non-cell associated manner, it is considered to be a Type II virus strain.

FIPV and FECV, TGEV, and CCV were passaged two or three times in fcwf-4 cells, CPK cells, and CrFK cells, respectively, and were used for the study.

Preparation of virus antigen. The antigen was prepared with the FIPV 79-1146 strain or FECV 79-1683 strain grown in fcwf-4 cell cultures. Infectious culture fluid concentrated about tenfold by ammonium sulfate precipitation was layered onto a discontinuous sucrose density gradient (20 and 60%) in an RPS 28 rotor (Hitachi Koki Co., Ltd., Japan) and centrifuged at 27 000 r.p.m. for 2 h. The virus bands formed were collected, diluted in NTE buffer, (0.1 M NaCl, 0.01 M Tris–HCl, pH 7.4, 0.001 M EDTA) and centrifuged at 80 000 *g* for 1 h. The virus-containing pellet was suspended in a 1/500 volume of NTE buffer.

Production of antibody-secreting hybrydomas. BALB/c mice, about 5 weeks of age, were inoculated intraperitoneally with a mixture of 50 μg of the viral antigen prepared as above and 10^9 cells of pertussis adjuvant. Four or six weeks later the mice received an intravenous booster dose of 50 μg of viral antigen, and spleen cells were obtained for fusing 3 d later. The fusion was carried out by essentially the same method described by Köhler and Milstein (1975). Polyethyleneglycol-4000 (Merck, Germany) was used as a fusing agent and the ratio of mouse spleen cells and mouse myeloma cells (P-3/X-63-Ag8-6,5,3) was 10:1. The selective medium contained hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M) and thymidine (1.6×10^{-5} M). The fused cells at a concentration of 3.5×10^6 spleen cells per ml was dispensed in 100 μl volumes into wells of 96-well, flat-bottomed microplates (Corning Glass Works, Corning, NY) and incubated at 37°C in a humid atmosphere containing 5% CO_2. After incubation for 2 weeks, the wells were examined and those which contained hybridoma cultures were tested for feline coronavirus specific antibody by an indirect immunofluorescence test (see below). The colonies in antibody positive wells were passaged in 24-well multiplates (Corning Glass Works, Corning, NY) and incubated in medium containing hypoxanthine (10^{-4} M) and thymidine (1.6×10^{-5} M). The cells were then cloned by the soft agar method.

Determination of antibody class and subclass. The supernatant fluids of antibody-secreting hybridoma cultures were concentrated tenfold by 50% saturation with ammonium sulfate and used for determination of antibody class and subclass by double diffusion in 1% agar gel containing 0.1% NaN_3. Rabbit antisera against mouse immunoglobulins, IgG 1, IgG 2a, IgG 2b, IgG 3, IgM and IgA, and κ and λ chains (Miles Laboratories, U.S.A.) were placed in center wells and test samples were added to adjacent wells. The plates were incubated overnight at room temperature in a humidified chamber.

Indirect fluorescent antibody assay. Hybridoma culture supernatant fluid was added to acetone-fixed infected monolayers, incubated for 30 min at 37°C, washed 3 times with phosphate buffered saline solution (PBS) and then stained with rabbit anti-mouse-IgG,A,M serum conjugated with fluorescein isothiocyanate (FITC) (Miles Lab., U.S.A.). After a further 30 min incubation at 37°C, slides were washed in PBS. Stained monolayers were mounted in buffered glycerol and examined using a fluorescence microscope.

Neutralization (NT) test. Serial twofold dilution of the MAbs were mixed with an equal volume of a virus suspension diluted so as to contain approximately 200 $TCID_{50}$/0.1 ml. The mixtures were incubated at 37°C for 60 min. Each mixture was then inoculated into cell cultures in flat-bottomed microplates, and incubated in an atmosphere of 5% CO_2 in air at 37°C for 6 d. Two wells

were employed for each antibody dilution. The antibody titer was expressed as the reciprocal of the highest dilution of MAb that completely inhibited cytopathic effect in the test.

Western immunoblotting. Viral antigen separated in polyacrylamide gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) were transferred to nitrocellulose sheets of 0.45 μm pore size. The transfer was carried out electrophoretically by the method adapted from Towbin et al. (1979) in a Transfer-Blot cell apparatus at 120 mA and 10 V for 14 h using transfer buffer consisting of 3 g l^{-1} Tris (pH 8.3), 20% methanol and 43.2 g l^{-1} glycine. The nitrocellulose sheets were then cut into strips and incubated at 37°C for 2 h in PBS containing 10% fetal-calf serum. The supernatant fluid of antibody-secreting hybridoma cultures was added in 1 ml volumes to individual strips and incubated at 37°C for 2 h. The strips were then washed 3 times with PBS containing 0.05% Tween-20, and incubated at 37°C for 2 h with horseradish peroxidase-conjugated rabbit antibody against mouse IgG,A,M (Miles Lab., U.S.A.) diluted 1:300 with PBS containing 10% fetal calf serum. The strips were then washed and treated with substrate solution containing 0.05 g diaminobenzidine, 50 μl of 30% H_2O_2 in 100 ml of 0.05 M Tris–HCl, pH 7.2. When distinct bands appeared about 10 min later, the reaction was stopped by pouring off the substrate solution and rinsing with distilled water.

Determination of polypeptide specificity by enzyme-linked immunosorbent assay (ELISA). The polypeptide specificity of the MAbs which could not be determined by western immunoblotting was determined according to the method of Fiscus and Teramoto (1987). Briefly, the virus antigen described above was first disrupted with 1% Nonidet P-40 (NP-40). This material was placed on a 15–50% linear sucrose density gradient containing 0.1% NP-40, and centrifuged at 80 000 *g* for 17 h. After fractionation, each fraction was diluted with NTE buffer, and allowed to be absorbed by 96-well, flat-bottomed Microelisa plates. The MAbs against N, E1 and E2 proteins with polypeptide specificity clarified by western immunoblotting were delivered into the wells of each fraction, and subjected to ELISA. ELISA was performed according to the method of Hohdatsu et al. (1987). Among the MAbs which recognize each protein, fractions which reacted strongly with a single type of MAb were collected. ELISA was performed with these fractions used as antigens, and the polypeptide specificity of the MAbs was determined.

RESULTS

Isolation and characterization of monoclonal antibodies. When FIPV strain 79-1146 was used as the immunogen, 25 MAbs (F2-1, F29-1, F16-4, F18-2, F19-1, F30-1, F34-1, F35-2, F36-1, F41-1, F51-1, F52-1, F70-2, F75-3, F15-

2, F24-1, F25-1, F46-4, F49-1, F69-3, F80-1, F6-3, F22-3, F23-2, F50-4) were obtained. In addition, with FECV strain 79-1683 as the immunogen, 6 MAbs (E15-2, E19-1, E22-2, E6-2, E25-2, E-12-1) were obtained. The polypeptide specificity, Ig isotypes, and NT activity to FIPV strain 79-1146 of these MAbs are shown in Table 2. For most MAbs, western immunoblotting could deter-

TABLE 2

Polypeptide specificity, Ig isotype, and neutralization (NT) activity to FIPV strain 79-1146 of monoclonal antibodies

MAb no	Polypeptide[1] specificity		Ig isotype	NT titer
	Blot	ELISA		
F 2-1	N	N	IgG1·K	<2
F 29-2	N	N	IgG1·K	<2
E 15-1	N	N	IgG1·K	<2
E 19-1	N	N	IgG1·K	<2
E 22-2	N	N	IgG1·K	<2
E 6-2	N	N	IgG1·k	<2
F 16-4	E1	E1	IgG3·K	<2
F 18-2	E1	E1	IgG1·K	<2
F 19-1	E1	E1	IgG1·K	<2
F 30-1	E1	E1	IgG3·K	<2
F 34-1	E1	E1	IgG2a·K	<2
F 35-2	E1	E1	IgG3·K	<2
F 36-1	E1	E1	IgG3·K	<2
F 41-1	E1	E1	IgG1·K	<2
F 51-1	E1	E1	IgG2a·K	<2
F 52-1	E1	E1	IgG2a·K	<2
F 70-2	E1	E1	IgG2a·K	<2
F 75-3	E1	E1	IgG2a·K	<2
F 15-2	E2	E2	IgG1·K	<2
F 24-1	E2	E2	IgG2a·K	<2
F 25-1	E2	E2	IgG2a·K	<2
F 46-4	E2	E2	IgG2a·K	<2
F 49-1	E2	E2	IgG2a·K	<2
F 69-3	E2	E2	IgG1·K	64
F 80-1	E2	E2	IgG1·K	<2
F 6-3	?	E2	IgG1·K	<2
F 22-3	?	E2	IgG2a·K	<2
F 23-2	?	E2	IgG2a·K	<2
F 50-4	?	E2	IgG2b·K	<2
E 25-2	E2	ND[2]	IgG2b·K	<2
E 12-1	E2	ND	IgM·K	256

[1]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 SDS–PAGE or by ELISA. For the ELISA, the three structural components of FIPV were separated by sucrose gradient centrifugation of detergent-disrupted FIPV virions.
[2]Not done.

mine their polypeptide specificity. However, this method failed to determine the specificity of 4 MAbs, F6-3, F22-3, F23-2 and F50-4. ELISA using NP-40 disrupted, sucrose gradient-purified viral polypeptide revealed that these MAbs recognise E2 protein. Moreover, western immunoblotting and ELISA using viral polypeptide yielded the same results with respect to the other MAbs. Figure 1 shows examples of the western immunoblotting reaction of MAbs which recognize N, E1 and E2 proteins. As shown in Table 2, two (F69-3, E12-1) of the 31 MAbs had NT activity to FIPV strain 79-1146.

Reactivity of the monoclonal antibodies with the feline coronaviruses. We examined the MAbs for reactivity with feline coronaviruses by IFA. As shown in Table 3, the MAbs could be divided into six groups according to their reactivity with 11 strains of feline coronavirus. The 16 MAbs in Group I all reacted with the feline coronaviruses. The two MAbs in Group II failed to react with two of the virus strains, while the one MAb in Group III showed no reaction with four strains. These MAbs were all found to recognize the N protein of the viruses. MAbs in Group IV reacted with two strains of FIPV and the one strain of FECV, while Group V MAbs reacted only with two strains of FIPV. The two MAbs in Group VI did not react with any FIPV strain but

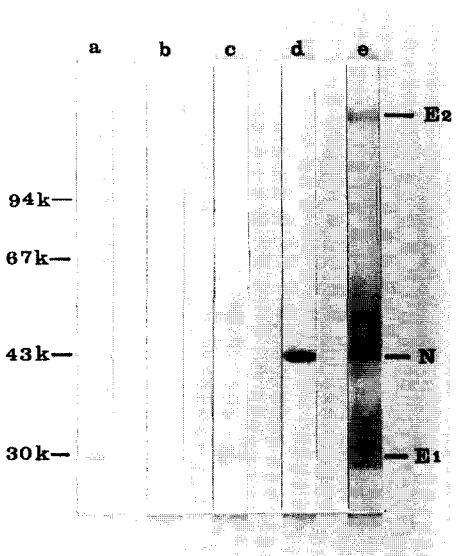

Fig. 1. Polypeptide specificity of monoclonal antibodies against FIPV 79-1146 strain in western immunoblotting. SDS–PAGE was performed on 10% gel under non-reducing conditions before immunoblot. a: marker proteins, b: F 25-1 (anti E2), c: F 70-2 (anti E1), d: F 2-1 (anti N), e: anti-FIPV mouse serum.

TABLE 3. Reactivity of the monoclonal antibodies to the feline coronaviruses

Group	MAb no	FIPV										FECV	Polypeptide specificity
		Type II		Type I								Type II	
		79-1146	KU-1	UCD-1	NW-1	Yayoi	KU-2	UCD-2	Black	UCD-3	UCD-4	79-1683	
I	F 2-1	++	++	++	++	+	+	++	++	+	++	++	N
	F 16-4	++	++	++	+	++	++	+	++	+	+	++	E1
	F 18-2	++	++	++	+	++	++	+	++	+	+	++	E1
	F 19-1	++	++	++	+	++	++	+	++	+	+	++	E1
	F 30-1	++	++	++	+	++	+	++	+	+	++	+	E1
	F 34-1	++	++	++	+	+	++	++	++	+	+	+	E1
	F 35-2	+	++	++	+	+	++	++	+	+	+	++	E1
	F 36-1	+	++	++	+	+	++	++	++	+	+	++	E1
	F 41-1	+	++	++	+	++	++	++	++	+	+	++	E1
	F 51-1	++	++	++	+	+	++	+	++	++	+	++	E1
	F 52-1	++	++	++	+	++	+	+	++	+	+	+	E1
	F 70-2	++	++	+	+	+	+	++	++	+	+	+	E1
	F 75-3	++	++	++	+	+	++	++	++	+	+	++	E1
	F 80-1	++	++	+	+	+	+	+	+	+	+	+	E2
	E 15-1	++	++	+	++	++	++	++	++	+	++	++	N
	E 19-1	++	+	+	++	+	++	++	++	+	++	+	N
II	E 6-2	++	++	++	+	+	+	++	++	–	–	++	N
	E 22-2	++	++	++	++	+	+	++	++	–	–	+	N
III	F 29-2	++	++	+	+	+	+	–	–	–	–	++	N
IV	F 50-4	+	+	–	–	–	–	–	–	–	–	+	E2
	F 46-4	++	++	–	–	–	–	–	–	–	–	+	E2
	F 69-3	++	++	–	–	–	–	–	–	–	–	+	E2
	F 15-2	++	++	–	–	–	–	–	–	–	–	+	E2
V	F 25-1	+	+	–	–	–	–	–	–	–	–	–	E2
	F 22-3	++	++	–	–	–	–	–	–	–	–	–	E2
	F 23-2	++	++	–	–	–	–	–	–	–	–	–	E2
	F 24-1	++	++	–	–	–	–	–	–	–	–	–	E2
	F 49-1	++	++	–	–	–	–	–	–	–	–	–	E2
	F 6-3	++	++	–	–	–	–	–	–	–	–	–	E2
VI	E 25-2	–	–	–	–	–	–	–	–	–	–	+	E2
	E 12-1	–	–	–	–	–	–	–	–	–	–	+	E2

By means of indirect IF test, with FITC-conjugated rabbit anti-mouse immunoglobulin, on each virus-infected fcwf-4 cell culture grown on coverslips. The monoclonal antibodies were used undiluted cell culture fluids. The minus sign indicates negative reactivity. 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 SDS–PAGE or by ELISA.

TABLE 4

Reactivity of the monoclonal antibodies to porcine and canine coronaviruses

Group	MAb no	CCV	TGEV		Polypeptide specificity
		1-71	SH	TO-163	
I	F 2-1	+	+	+	N
	F 16-4	++	++	++	E1
	F 18-2	++	++	++	E1
	F 19-1	++	++	++	E1
	F 30-1	++	++	++	E1
	F 34-1	++	++	++	E1
	F 35-2	++	++	++	E1
	F 36-1	++	++	++	E1
	F 41-1	++	++	++	E1
	F 51-1	++	++	++	E1
	F 52-2	++	++	++	E1
	F 70-1	++	++	++	E1
	F 75-3	++	++	++	E1
	F 80-1	+	++	+	E2
	E 15-1	++	++	++	N
	E 19-1	++	++	+	N
II	E 6-2	–	–	–	N
	E 22-2	–	–	–	N
III	F 29-2	–	–	–	N
IV	F 50-4	+	–	–	E2
	F 46-4	+	++	+	E2
	F 69-3	+	++	+	E2
	F 15-2	–	–	–	E2
V	F 25-1	–	–	–	E2
	F 22-3	–	–	–	E2
	F 23-2	–	–	–	E2
	F 24-1	–	+	–	E2
	F 49-1	–	+	–	E2
	F 6-3	++	–	–	E2
VI	E 25-2	+	–	–	E2
	E 12-1	+	–	–	E2

By means of indirect IF test, with FITC-conjugated rabbit anti-mouse immunoglobulin, on each virus-infected CvFK or CPK cell culture grown on coverslips. The monoclonal antibodies were used undiluted cell culture fluids. The minus sign indicates negative reactivity.
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 SDS–PAGE or by ELISA.

did react with the only strain of FECV. All MAbs in Group IV, V and VI were found to recognize E2 protein.

Reactivity of the monoclonal antibodies with porcine and canine coronaviruses. We examined the MAbs for reactivity with porcine and canine coronaviruses by IFA. Table 4 shows the results. All MAbs in Group I, which reacted with all feline coronaviruses, also reacted with CCV and TGEV. MAbs in Groups II and III, which did not react with UCD-2, UCD-3, UCD-4 and Black strains, did not react with CCV and TGEV either. However, out of four MAbs in Group IV, which reacted only with the 79-1146 and KU-1 strains of FIPV and strain 79-1683 of FECV, three reacted with either CCV or TGEV. Moreover, among the MAbs in Group V, which reacted only with the 79-1146 and KU-1 strains of FIPV, two reacted with the SH strain of TGEV, and 1 type reacted with the 1-71 strain of CCV. Furthermore, MAbs in Group VI, which reacted with FECV alone, reacted with the 1-71 strain of CCV.

DISCUSSION

Thirty-one MAbs were prepared by using strain 79-1146, classified as FIPV Type II, and strain 79-1683, classified as FECV Type II, as immunogens. Table 3 shows their reactivity with 11 strains of feline coronavirus. All 16 MAbs in Group I reacted with feline coronaviruses. Besides feline coronaviruses, these MAbs reacted with the 1-71 strain of CCV, and the SH and TO-163 strains of TGEV. These results confirm the previous reports (Pedersen et al., 1978; Horzinek et al., 1982; Pedersen et al., 1984a) that these virus strains are antigenically close to each other. Concerning polypeptide specificity, many MAbs in this Group I recognize E1 protein. Among these viruses, many common epitopes seem to exist, particularly in the E1 protein. Among eight virus strains with the characteristics of FIPV Type I, reactivities of E6-2 and E22-2 in Group II, and F29-2 in Group III with the MAbs were different, and all of these MAbs recognized N protein. Four MAbs in Group IV (F50-4, F46-4, F69-3 and F15-2) reacted with the 79-1146 and KU-1 strains of FIPV Type II and strain 79-1683 strain of FECV Type II, but not with FIPV Type I viruses. Of these MAbs, however, F50-4 reacted with CCV, and F46-4 and F69-3 reacted with CCV and TGEV. Similarly, among the MAbs in Group V, which react with FIPV Type II viruses alone, F6-3 reacted with CCV, and F24-1 and F49-1 reacted with the SH strain of TGEV. Furthermore, all MAbs in Group VI, which react with the 79-1683 strain of FECV alone reacted with CCV. As Pedersen et al. (1984a) have reported from their study with polyclonal antibody, these results suggest that FIPV Type II viruses and FECV Type II viruses are antigenically closer to TGEV or CCV than to FIPV Type I viruses. At present, the authors are preparing MAbs with neutralizing activity using FIPV strain 79-1146 as immunogen, and are determining the serological re-

lationships among these viruses by the presence or absence of the neutralization epitope. Moreover, since all 12 MAbs in Groups IV, V and VI recognize E2 protein, it was assumed that there are many antigenic differences in E2 protein among these viruses. By using MAbs, Fiscus and Teramoto (1987) also found antigenic differences among feline coronaviruses, especially in the E2 protein.

Conventional serological methods have failed to discriminate between strain 79-1146 of FIPV Type II and strain 79-1683 of FECV Type II. However, MAbs in Group V reacted with FIPV Type II viruses alone, while MAbs in Group VI reacted with the 79-1683 strain of FECV Type II alone. These MAbs have enabled the discrimination of these viruses, by clearly indicating antigenic differences among them. The proportions of Types I and II of FIPV and Types I and II of FECV actually present in the natural environment are not clear. As stated in the introduction, the neutralizing test can appraise infection with Type I and II of FIPV at the serological level. However, in the case of infection with viruses other than FIPV Type I, distinction of infection particularly by FIPV Type II an FECV Type II at the serological level is difficult. In the future, it will be of use to be able to distinguish infection of these viruses by competitive enzyme immunoassay (Fiscus et al., 1985; Fiscus et al., 1987) using type-specific MAbs. The MAbs in Groups V and VI which the authors have prepared in this study are expected to be useful as such type-specific MAbs.

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