Production of a Monoclonal Antibody Reacted Broadly with Feline Calicivirus Field Isolates

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ABSTRACT. A monoclonal antibody (MAb) reactive with 36 field isolates and 2 laboratory strains of feline calicivirus (FCV) was produced by immunizing mice with the mixture of FCVs. The MAb (4D7) reacted with FCVs in an enzyme-linked immunosorbent assay (ELISA), but had no neutralizing activity against the F4 strain of FCV. MAb 8G1, previously produced against the FCV F4 strain, also reacted in ELISA with all FCVs used in the present study. However, the epitopes recognized by 4D7 and 8G1 were different. Using these two MAbs and a polyclonal rabbit antibody, we attempted to develop a sandwich ELISA for detection of FCV antigen. The combination of 4D7 and the polyclonal rabbit IgG was most sensitive. Using this system, all the field isolates of FCV cultured *in vitro* were detected. However, among the 36 swab samples, from which FCV was isolated, 4 were negative.—KEY WORDS: feline calicivirus, monoclonal antibody, sandwich ELISA.

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Feline calicivirus (FCV) is one of the major pathogens for cat upper respiratory diseases. Although vaccination for FCV has been available for almost twenty years, a high proportion of vaccinated cats present with clinical signs of FCV infection [1]. A number of different strains of FCV have been isolated and the serological relationships among these isolates have been investigated since 1970's using polyclonal antibodies [2–4, 6, 7] and monoclonal antibodies [5, 12]. The most of previous reports analyzed the serological differences among FCV isolates and there are few reports about the antigen conserved widely among the FCV isolates [12]. Characterization of the common antigens of FCVs is important for the development of effective vaccine and the detection of FCV antigen. In the present study, we attempted to produce a monoclonal antibody (MAb), which could react broadly with FCV field isolates, and examined its application to an enzyme-linked immunosorbent assay (ELISA) for the detection of FCV antigen.

MATERIALS AND METHODS

Cells and viruses: Crandell feline kidney (CRFK) cells were used for propagation of the viruses. Field isolates of FCV were isolated from the oral or nasal swabs of cats suspected of having viral respiratory diseases [9]. The swab samples used for the virus isolation were stored at −80°C and used for FCV antigen detection in the present study. The F4 strain of FCV was isolated in 1968 and is the prototype strain of FCV in Japan [11]. The F9 strain is a vaccine strain and widely used in Japan. All the field isolates were purified by three rounds of plaque cloning and propagated in CRFK cells.

Serial passages of 9 field isolates, F4 and F9 strains were carried out and the high-passaged viruses, passage number of 100, were also used.

The 90–8A strain of FHV1, isolated in 1990 from a cat with respiratory disease, was also used in the present study.

Preparation of virus antigen: Virus antigen for immunization and ELISA was prepared from culture fluid of CRFK cells inoculated with each strain of FCV. The culture fluid of FCV was collected and centrifuged at 1,000G for 10 min. For precipitation of the virus, 22.8 g of ammonium sulfate was added to 100 m*l* of the culture fluid. After centrifugation at 10,000G for 20 min, the virus was suspended in Dulbecco's phosphate buffered saline (PBS) and dialyzed overnight against PBS at 4°C. Following centrifugation at 700G, the protein concentration was measured by using the Bio Rad protein assay (Bio Rad Laboratories, Inc., CA, U.S.A.). For immunization, four types of antigen were prepared. Antigen A was a mixture of all strains of FCV. Antigen B and antigen C were each a mixture of 13 strains of FCV field isolates. Antigen D was a mixture of 10 field isolates, together with the F4 and F9 strains of FCV.

Anti-FCV monoclonal antibodies: Six week-old BALB/cCrSlc mice were immunized with antigen A intraperitoneally followed by a second immunization with antigen A subcutaneously 1 week later. The mice were then immunized subcutaneously with antigen B, C and D at 5 day intervals. The last immunization was performed subcutaneously using antigen A. Three days after the last immunization, the mice were killed and the spleen cells fused with the myeloma cells, P3X63Ag8.653 using polyethylene glycol 1,500 (Boehringer Mannheim GmbH, Germany). Hybridomas were selected using HAT medium

and screened for antibody production by ELISA. As antigens for the negative control, FHV1, CRFK cells disintegrated by ultrasonication and fetal calf serum were used. One hybridoma secreted the antibody 4D7 that reacted with all the FCV used in this study. The hybridoma was cloned by the limiting dilution method and the immunogloblin isotype of antibody was determined by ELISA using the Mouse Typer Sub-Isotyping Kit (Bio-Rad Laboratories, U.S.A.). The culture supernatant and ascitic fluid from the mice were used as antibody in the present study.

MAb 8G1 and 16E8 raised against the F4 strain of FCV were also used. The antibody 8G1 reacted with FCV in ELISA and western blotting, but had no neutralizing activity [8]. MAb 16E8 reacted with FCV F4 strain in ELISA and neutralized FCV Japanese isolates [12]. Ascitic fluids from mice were used as antibodies in the present study.

The IgG fraction was purified from 4D7 and 8G1 using MabTrapG (Pharmacia LKB Biotechnology Inc., U.S.A.).

Anti-FCV polyclonal antibody: Anti-FCV polyclonal antibody was prepared in a rabbit. First, the antigen A was inoculated intravenously and then an antigen mixture of 20 field isolates was given intramuscularly 2 weeks later. After a further 2 weeks an antigen mixture consisting of 16 field isolates, together with the F4 and F9 strains was inoculated. Finally antigen A was inoculated intravenously and after 1 week, the blood was collected by heart puncture. IgG fraction was purified by protein A affinity chromatography (E-Y Laboratories, Inc., U.S.A.) from the antiserum.

Immunoblot analysis: For immunoblot analysis, the FCV F4 strain was used as an antigen. The antigen was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using a 10% gel, and then transferred to nitrocellulose membrane. The immunostaining was carried out using 4D7 and the Vectastain ABC-PO (mouse IgG) kit (Vector Laboratories, Inc., U.S.A.) or a polyclonal rabbit IgG and the Vectastain ABC-PO (rabbit IgG) kit (Vector Laboratories, Inc., U.S.A.). The colorimetric reaction was done by the addition of 0.2 mg/m*l* 4-chloro-1-naphthol in PBS containing 16.6% methanol and 0.015% H_2O_2.

Neutralization test: Culture fluid of 4D7 was used for the neutralization test. Twofold serial dilutions of 4D7 starting from 1:1 to 1:128 were prepared. The dilutions were mixed with an equal volume of culture fluid from FCV-infected CRFK cells containing 2×10^3 $TCID_{50}$/m*l*, and incubated at 37°C for 1 hr. The mixture was then inoculated into CRFK monolayer cells seeded on microplate wells. After cultivation for 3 days, the results were read. The tests were carried out twice.

ELISA: FCV antigen diluted with 0.05 M carbonate buffer (pH 9.6), at the protein concentration of 10 μg/m*l*, was adsorbed onto individual wells of microplates and left overnight at 4°C. Excess antigen was removed and the wells were then coated with 100 μl of 25% Block Ace (Dainippon Pharmaceutical Co., Ltd., Japan) in PBS (PBS-Block Ace) for 60 min at 37°C to block non-specific protein adsorption sites. The antibody was added to duplicate wells (50 μl/well) and reacted with the antigen for 60 min at 37°C. After 3 washes with 0.05% Tween 20 in PBS, 50 μl of a 1:1,000 dilution of peroxidase-conjugated anti-mouse IgG goat serum (Bio Source International, U.S.A.) or 1:500 dilution of peroxidase-conjugated anti-rabbit IgG goat serum (Bio Source International, U.S.A.) was added to each well and incubated for 30 min at 37°C. The wells were again washed as described above and the colorimetric reaction was carried out by the addition of 0.2 mM 2,2-azinobis(3-ethylbenzthiazoline-sulfonic acid) and 0.004% H_2O_2 in 0.05 M citrate buffer, pH 4.0, as substrate. After incubation for 15 min, the absorbance at 405 nm was measured. For the negative control, ascitic fluid from mice inoculated with SP2/O cells or normal rabbit serum was used. The absorbances of the control wells were subtracted from those of the test wells and if the resulting absorbance was higher than 0.1, it was considered positive. The ELISA titer was expressed by the reciprocal of the highest dilution that showed a positive reaction. The test was done twice and geometric means were calculated.

Competitive binding assay: The competitive binding assay using 4D7 and 8G1 was performed by ELISA using the avidin-biotin system. One mg of purified IgG from MAb in 1 *ml* of PBS was mixed with 20 μl of a 50 mg/m*l* solution of biotinyl-N-hydroxysuccinimide ester (E-Y Laboratories, Inc., U.S.A.) and incubated at room temperature for 3 hr. After incubation, the mixture was dialyzed overnight against PBS at 4°C. The antigen used for each antibody was the FCV F4 strain and it was prepared in the same manner as described above. Antigen-coated plates were prepared as described above. After blocking with PBS-Block Ace, serial 2-fold dilutions of each competitor were added to the wells. After incubation at 37°C for 1 hr, the wells were washed as described above, and biotin-labeled IgG diluted to 1:200 was added. After incubation, a 1:500 dilution of ExtrAvidin-Peroxidase (Sigma Chemical Company, U.S.A.) and substrate were added sequentially as described above and then the absorbance at 405 nm was measured.

Sandwich ELISA: For detection of FCV antigen, a sandwich ELISA was examined using combinations of 4D7, 8G1 and a polyclonal rabbit IgG. As antibodies, the 4D7 and 8G1 were used at a 1:10,000 dilution of ascitic fluid from mice and the polyclonal rabbit IgG was used at the protein concentration of 10 μg/m*l*. The capturing antibody was prepared by using 0.05 M carbonate buffer, pH9.6, and coated onto a 96 well plate. The plate was stored at 4°C overnight and recoated with PBS-Block Ace for 30 min at 37°C. Fifty μl of the specimen was added to each well and incubated for 1 hr at 37°C. After washing, 50 μl of the detecting antibody prepared in PBS-Block Ace was added and incubated for 1 hr at 37°C. In cases where both antibodies were MAbs, the detecting antibody was used after biotinization in the same manner as described above and at the protein concentration of 10 μg/m*l*. After further washing, 50 μl of the conjugate was added to each well. The conjugate was selected from peroxidase-conjugated anti-rabbit IgG goat serum (1:500 dilution, Bio Source

International, U.S.A.), peroxidase-conjugated anti-mouse IgG goat serum (1:500 dilution, Bio Source International, U.S.A.) and ExtrAvidin-Peroxidase (1:500 dilution, Sigma Chemical Company, U.S.A.) according to the second antibody. The colorimetric reaction was carried out as described in the ELISA technique.

RESULTS

Production and characterization of 4D7: Three cycles of spleen cell-myeloma fusion were performed and 94 antibody-secreting hybridomas were obtained. Among them, only one (4D7) secreted antibody that reacted with all the FCV strain used. MAb 4D7 was an IgG_1 (κ) type antibody. As shown in Table 1, 4D7 reacted with all the field isolates, and the F4 and F9 strains with a range of titer from 1:320 to more than 1:2,560. No reactivity of the antibody was observed against FHV1, CRFK cells disintegrated by ultrasonication or fetal calf serum (data not shown). MAb 4D7 did not neutralize the FCV F4 strain. From the results of immunoblot analysis, it was found that 4D7 recognized a 67 kDa molecule (Fig. 1).

Reactivities of 8G1 and a rabbit polyclonal antibody with FCVs: Reactivities of 8G1 and a rabbit polyclonal antibody with various strains of FCV are shown in Table 1. MAb 8G1 reacted strongly with many FCV field isolates. However, the reaction with 4 field isolates was extremely weaker than the other strains. The rabbit polyclonal IgG reacted with all the strains and the titer ranged from 1:8,000 to more than 1:64,000.

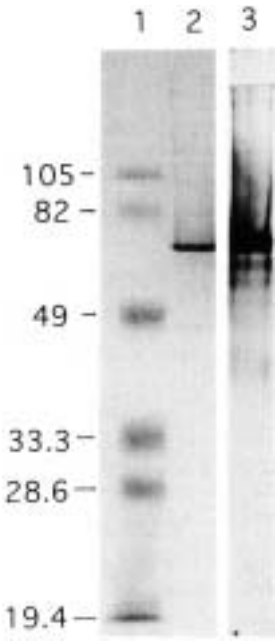

Fig. 1. Immunoblot analysis with monoclonal antibody 4D7. Lane 1: marker. Lane 2: feline calicivirus F4 strain stained with monoclonal antibody 4D7. Lane 3: feline calicivirus F4 strain stained with anti-FCVs polyclonal antibody.

Reactivities of MAbs and polyclonal IgG with the FCV high-passaged strain: Table 2 shows the reactivity of the MAbs and polyclonal IgG with FCVs of passage number 2 and 100. The reactivity of 16E8 with FCV 91–1, 91–25, 91–43 and F4 changed drastically during serial passages. However, 4D7 and 8G1 consistently reacted with each passage number of FCV as did the polyclonal IgG.

Competitive binding assay: Figure 2 shows the results of the competitive binding assay with 4D7 and 8G1. The reactivity of each antibody was blocked by the homologous competitor but not by the heterologous competitor, indicating that 4D7 and 8G1 recognized different epitopes.

Sandwich ELISA: To establish a sandwich ELISA system

Table 1. Reactivities of anti-feline caliciviruses (FCVs) monoclonal and polyclonal antibodies with various FCV strains

FCV strain	Monoclonal antibodies 4D7[a)]	Monoclonal antibodies 8G1[b)]	Polyclonal IgG[c)]
91– 1	≧ 2,560[d)]	≧ 1,280,000	32,000
91– 2	≧ 2,560	≧ 1,280,000	32,000
91– 3	≧ 2,560	≧ 1,280,000	32,000
91– 5	≧ 2,560	≧ 1,280,000	≧ 64,000
91– 6	1,280	≧ 1,280,000	32,000
91– 7	1,280	10,000	32,000
91– 9	1,280	10,000	32,000
91–10	≧ 2,560	≧ 1,280,000	32,000
91–11	1,280	≧ 1,280,000	32,000
91–12	1,280	≧ 1,280,000	32,000
91–13	640	≧ 1,280,000	≧ 64,000
91–14	≧ 2,560	≧ 1,280,000	32,000
91–16	≧ 2,560	≧ 1,280,000	≧ 64,000
91–17	≧ 2,560	≧ 1,280,000	≧ 64,000
91–18	≧ 2,560	≧ 1,280,000	32,000
91–19	1,280	≧ 1,280,000	32,000
91–20	≧ 2,560	≧ 1,280,000	≧ 64,000
91–21	1,280	≧ 1,280,000	≧ 64,000
91–22	≧ 2,560	≧ 1,280,000	≧ 64,000
91–24	1,280	≧ 1,280,000	32,000
91–25	320	≧ 1,280,000	8,000
91–26	320	≧ 1,280,000	16,000
91–27	1,280	≧ 1,280,000	32,000
91–28	640	≧ 1,280,000	8,000
91–29	640	≧ 1,280,000	32,000
91–31	640	≧ 1,280,000	16,000
91–32	640	≧ 1,280,000	32,000
91–33	640	10,000	≧ 64,000
91–34	640	≧ 1,280,000	32,000
91–35	1,280	≧ 1,280,000	32,000
91–36	1,280	≧ 1,280,000	16,000
91–37	≧ 2,560	10,000	32,000
91–38	1,280	≧ 1,280,000	NT
91–40	1,280	≧ 1,280,000	≧ 64,000
91–41	1,280	≧ 1,280,000	32,000
91–43	≧ 2,560	≧ 1,280,000	≧ 64,000
F4	1,280	≧ 1,280,000	16,000
F9	1,280	≧ 1,280,000	32,000

a) Supernatant of hybridoma was used as an antibody. b) Ascitic fluid of mice was used as an antibody. c) IgG from anti-FCVs rabbit serum. d) Reciprocal of the highest dilution of the antibody that indicate positive.

Table 2. Reactivities of monoclonal and polyclonal antibodies with various passage of feline caliciviruses (FCV)

FCV strain	Passage No.	Monoclonal antibody 4D7[a)]	8G1[b)]	16E8[b)]	Polyclonal IgG[c)]
91–1	2	≧ 2,560[d)]	≧ 1,280,000	≧ 1,280,000	32,000
	100	1,280	≧ 1,280,000	< 10,000	32,000
91–3	2	≧ 2,560	≧ 1,280,000	10,000	16,000
	100	640	≧ 1,280,000	< 10,000	32,000
91–5	2	≧ 2,560	≧ 1,280,000	10,000	16,000
	100	1,280	≧ 1,280,000	< 10,000	32,000
91–17	2	≧ 2,560	≧ 1,280,000	< 10,000	16,000
	100	≧ 2,560	≧ 1,280,000	< 10,000	32,000
91–19	2	1,280	≧ 1,280,000	< 10,000	16,000
	100	1,280	≧ 1,280,000	< 10,000	≧ 64,000
91–22	2	≧ 2,560	≧ 1,280,000	< 10,000	32,000
	100	640	≧ 1,280,000	< 10,000	≧ 64,000
91–25	2	320	≧ 1,280,000	320,000	32,000
	100	1,280	≧ 1,280,000	< 10,000	≧ 64,000
91–31	2	640	≧ 1,280,000	80,000	16,000
	100	1,280	≧ 1,280,000	< 10,000	≧ 64,000
91–43	2	≧ 2,560	≧ 1,280,000	≧ 1,280,000	16,000
	100	≧ 2,560	≧ 1,280,000	< 10,000	≧ 64,000
F4	2	1,280	≧ 1,280,000	160,000	32,000
	100	1,280	≧ 1,280,000	< 10,000	≧ 64,000
F9	2	1,280	≧ 1,280,000	< 10,000	32,000
	100	≧ 2,560	≧ 1,280,000	< 10,000	≧ 64,000

a) Supernatant of hybridoma was used as an antibody. b) Ascitic fluid of mice was used as an antibody. c) IgG from anti-FCVs rabbit serum. d) Reciprocal of the highest dilution of the antibody that indicated positive.

for FCV antigen detection, a combination of 4D7, 8G1 and polyclonal rabbit IgG was examined. The minimum concentration of antigen detected by each system is summarized in Table 3. The combinations of 4D7 and rabbit IgG; 4D7 and 8G1; and 8G1 and rabbit IgG could detect the FCV F4 strain at protein concentrations lower than 3.125 μg/m*l*. From among these combinations, the absorbance at 405 nm was highest in the combination of 4D7 (capturing antibody) and rabbit IgG (detecting antibody) and therefore, this combination was used in further studies. The minimum titer of FCV detected by the sandwich ELISA is shown in Table 4. Although all the field isolates were detected in this system, the minimum titer of virus detectable varied from 0.5 to 7.0 $TCID_{50}$/m*l*. The FHV1 90-8A strain did not react in the sandwich ELISA (data not shown).

Detection of FCV antigen in swabs: The results of the FCV antigen detection using swabs from cats are summarized in Table 4. Among the 36 swab samples, from which FCV was isolated, 32 were FCV antigen positive. When the virus titer at the isolation was compared with minimum virus titer detectable, 21 samples showed a lower titer. However the FCV antigen could not be detected in only 3 samples. This indicated that sandwich ELISA used here could detect both infectious virus and virus antigen in the swab samples. When virus isolation, both FCV and FHV1 were isolated from 5 of these samples. In the present study, all of 5 samples were FCV antigen positive in sandwich ELISA. We also examined the swab samples

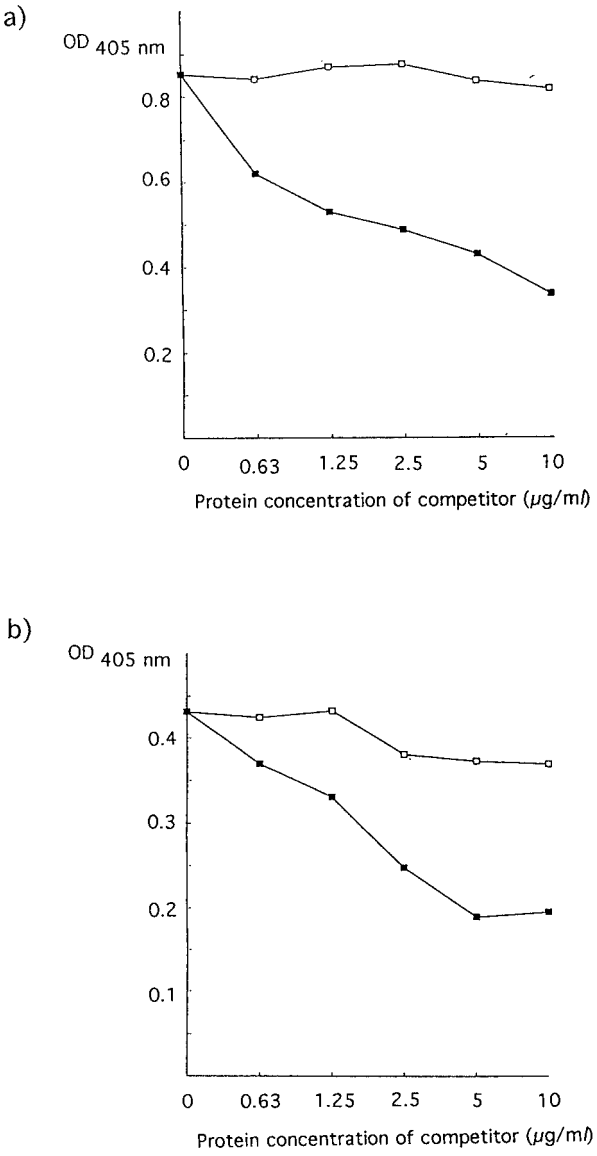

Fig. 2. Competitive binding assay of monoclonal antibodies 4D7 and 8G1 against feline calicivirus F4 strain. a) Biotin-labeled 4D7 and competitor 4D7 (■) or 8G1 (□). b) Biotin-labeled 8G1 and competitor 8G1 (■) or 4D7 (□).

from which only FHV1 or no virus was isolated. Of 30 FHV1-positive samples, none were positive in the sandwich ELISA. On the other hand, among the 30 virus-negative samples, 1 was positive in the sandwich ELISA. Although we again tried to isolate the virus from this sample, no virus was isolated (data not shown).

DISCUSSION

Antigenic differences of FCV strains were reported using not only conventional polyclonal antibodies but also MAbs [2–7, 12]. However there are few reports about the common antigens of FCV strains. Previously Tohya *et al.* reported that MAb 1D7 neutralized 20 FCV strains tested and they

Table 3. Minimum antigen concentration detectable in each sandwich ELISA system

Detecting antibody	Capturing antibody		
	Rabbit IgG	Mab 4D7	MAb 8G1
Rabbit IgG	NT[a)]	≦3.125[b)] (0.590)[c)]	≤ 3.125 (0.288)
MAb 4D7	25	NT	–[d)e)]
MAb 8G1	400	≦3.125[d)] (0.158)	–[d)e)]

a) Not tested. b) Protein concentration of FCV F4 strain (μg/m*l*). c) Difference of the absorbance with negative control. d) Second antibody was biotinized. e) Negative at the protein concentration of 400 μg/m*l*.

suggested that 1D7 recognized a common neutralizing epitope of FCV [12]. However our recent study revealed that many FCV field isolates did not react with 1D7 in the neutralization test [10]. As far as we know, there is no report about the production of a MAb which detects a common antigen of FCVs. In the present study, we attempted to produce MAb that reacted broadly with FCV field isolates. We supposed that the repeated immunization of mice with various isolates of FCV may give immunity to the mice against an antigen conserved widely among the FCVs. So we immunized mice with not only one strain but the mixture of 36 field isolates and 2 laboratory strains of FCV. After 3 rounds of fusion, we got 94 antibody-secreting hybridomas. However, only one (4D7) secreted the antibody that reacted with all FCVs in ELISA although this antibody could not neutralize the FCV F4 strain. At the same time, we examined the reactivity of MAb 8G1, which was previously produced against the F4 strain of FCV and has no neutralization activity [8]. We found that this antibody also recognized all the field isolates in ELISA. In the previous report, Shin *et al*. showed by immunoblot analysis that 8G1 recognized a 67 kDa capsid protein of FCV [8]. In the present study, 4D7 also recognized a 67 kDa molecule on immunoblot analysis. Although the molecule recognized appears the same, the results of the competitive binding assay revealed that the epitope recognized by each antibody was different.

Recently we found that the reactivities of neutralizing MAbs with FCV had changed during the serial passages of FCVs *in vitro* under the absence of antibodies (unpublished data). For example, the reactivity of 16E8 with some viruses changed during serial passages. So we examined the reactivities of 4D7 and 8G1 with high-passaged viruses. Both 4D7 and 8G1 reacted consistently with high-passaged viruses, indicating that the epitope recognized by each antibody is stable during serial passages of the virus. Previously Shin *et al*. examined the FCV capsid precursor protein produced in a mammalian cDNA expression system. They reported that 8G1 detected the 76 kDa capsid precursor protein in an immunoblot analysis. Furthermore 8G1 reacted with polypeptides expressed in COS-7 cells transfected with deleted or mutated cDNAs. These results indicated that 8G1 recognized amino acids encoded by RNA regions other than the variable region [8]. The present result of the constant reactivity of 8G1 with high-passaged FCV agree well with this previous report. However, concerning the epitope recognized by 4D7, further analysis is necessary.

The epitope, which is stable during the serial passages of the virus, might be useful for the antigen detection system. So we attempted to develop a sandwich ELISA system for the detection of the FCV antigen. A combination of antibodies for sandwich ELISA were examined and the combination of 4D7 and rabbit IgG was found to be the most sensitive. Using this system, all the field isolates of FCV cultured *in vitro* were detected. However the minimum titer of virus detectable varied. The reason of the variety in titer detectable could not be clarified in the present study.

Table 4. Comparison of the results from the sandwich ELISA using cultured feline calicivirus (FCV) and swab samples as antigens

FCV strain	Cultured virus	Swab sample	
	Minimum virus titer detected by sandwich ELISA	Virus titer at the isolation	Sandwich ELISA
91-1	6.0[a)]	4.5[a)]	+[b)]
91–2	5.5	4.5	+
91–3	4.0	3.0	+
91-5[c)]	6.0	2.0	+
91–6	5.0	4.5	+
91–7	6.5	2.0	+
91-9	0.5	3.5	+
91–10	0.5	4.0	+
91–11[c)]	3.5	3.5	+
91-12	6.5	2.5	–
91–13	4.5	2.0	+
91–14	4.0	4.0	–
91-16	5.0	4.0	+
91–17[c)]	7.0	4.0	+
91–18[c)]	3.0	3.0	+
91-19	1.0	3.0	+
91–20	0.5	3.5	+
91–21	0.5	2.0	+
91-22[c)]	0.5	1.5	+
91–24	6.0	2.0	+
91–25	5.5	2.5	+
91-26	5.5	3.0	+
91–27	4.5	2.0	+
91–28	4.5	2.0	+
91-29	1.0	2.5	+
91–31	4.0	3.0	+
91–32	0.5	4.5	+
91-33	5.0	3.0	–
91–34	3.0	2.0	+
91–35	3.0	2.5	+
91-36	2.5	2.0	–
91–37	4.0	5.0	+
91–38	2.0	3.0	+
91-40	5.5	4.5	+
91–41	4.0	2.5	+
91–43	1.0	4.5	+

a) Log $TCID_{50}$/m*l*. b) +; positive, –; negative. c) Both FCV and feline herpesvirus were isolated.

One possible explanation is that ELISA detects not only infective virus but also non-infectious virus antigen, as being mentioned later, and the virus antigen in cultured supernatant might affect the results. Further investigation about the non-infectious virus antigen in the cultured supernatant is necessary. The specificity of this system was revealed by the negative results obtained using cultured FHV1 and FHV1-positive swab samples. The application of swab samples from the mouth or nose of cats to the sandwich ELISA may be useful for clinical use. Therefore, we tried to use swab samples as antigens and 88.9% of the cat swab samples from which we isolated FCVs were positive for the FCV antigen on ELISA. Five samples which contained not only FCV but also FHV1 were positive on sandwich ELISA and no effect of the FHV1 was observed. Comparing virus titer at the isolation with the minimum virus titer detectable, the former titer was lower in 21 samples. However, the FCV antigen was still detected in 18 samples. This indicates that the sandwich ELISA detected not only infectious virus but also virus antigen which exists in the mouth or nose of infected cats and therefore, the virus antigen could not grow *in vitro*. This is also supported by the result that one swab sample was positive in the ELISA but was not positive for virus isolation.

In this study, the production of a MAb which reacted broadly with FCV field isolates and the development of an ELISA system for FCV antigen detection were shown. 4D7 reacted all the FCVs used in the present study and this is the first MAb produced for the purpose of detection of the common antigen of FCVs. It is unknown whether the epitopes recognized by 4D7 and 8G1 are really common to all the FCVs in the world or not. Further investigation about the regions of virus gene encoding these epitopes is necessary. Although this is the first report of an FCV antigen detection system, about 10% of the samples could still not be detected in the present study, indicating conventional virus isolation method is still more sensitive. Further improvements are necessary to make the system more sensitive for clinical use. In addition, analysis of the epitopes detected by 4D7 and 8G1 may be useful for the development of other antigen detection systems.

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