Epitope Mapping of a Monoclonal Antibody Specific to Feline Panleukopenia Virus and Mink Enteritis Virus

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ABSTRACT. To obtain monoclonal antibodies (MAbs) specific to feline panleukopenia virus (FPLV) and mink enteritis virus (MEV), 15 hybridomas secreting MAbs against MEV-Abashiri were established and the properties of the MAbs were analyzed. The cross-reactivity of MAbs revealed that one MAb, P2-215 was specific for FPLV and MEV, whereas the remaining fourteen MAbs reacted with canine parvovirus (CPV), FPLV, and MEV. Epitope analyses using various CPV/MEV chimeric viruses revealed that the MAb P2-215 recognized the epitope comprised of amino acid 93-Lys in VP2, which is known to be FPLV and MEV-specific. — KEY WORDS: canine parvovirus, feline panleukopenia virus, monoclonal antibody.

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Feline panleukopenia virus (FPLV), mink enteritis virus (MEV), and canine parvovirus (CPV) are classified as feline parvovirus host range variants of the genus Parvovirus, within the family *Parvoviridae* [14]. The antigenic and genetic similarities of CPV to FPLV and MEV, as well as the sudden emergence of CPV, strongly support the hypothesis that CPV is generated from FPLV or other related viruses by certain genetic mutations [11]. CPV differs from FPLV and MEV in some biological and genetic properties, whereas the only property that distinguishes FPLV from MEV is the host animal. Here we referred to FPLV and MEV as FPLV/MEV.

Recombinant virus studies revealed that only a few amino acid changes endow the host range of CPV to FPLV/MEV [2, 5]. CPV undergoes continuous antigenic change, which is thought to be caused by immune pressure [12], however, it is still unclear whether the appearance of CPV from FPLV or other related viruses is caused by the same antigenic mechanism. Recently, Truyen *et al.* suggested that selection for amino acid substitutions occurs in VP1/VP2, which is reflected in a high nonsynonymous/synonymous difference ratio in the VP1/VP2 gene [16]. The restrictive localization of the amino acid substitutions on the VP2 molecule [3, 16] may represent the existence of selection for functions of the capsid, such as viral infection and the formation of antigenic sites. Thus, analysis of monoclonal variants of FPLV/MEV should help uncover the mechanism of CPV appearance by clarifying whether immune pressure results in the appearance of the viruses that possess the CPV-like properties.

For the purpose of production of monoclonal variants of FPLV/MEV, we tried to establish hybridoma secreting monoclonal antibodies (MAbs) specific to FPLV/MEV. Here we describe the reactivities of MAbs, mapping of the epitope for FPLV/MEV-specific MAb, and discuss the utility of the FPLV/MEV-specific MAb.

The cells and viruses were propagated as described previously [4]. Mice were immunized with the MEV-Abashiri strain that had been purified using CsCl-isopycnic centrifugation. The splenocytes from the immunized mice were fused with myeloma cells P3-X63.Ag.8.653 or P3-X63.Ag.8.U1 by polyethylene glycol 1,500 according to the supplier's instructions (Boehringer Mannheim). Hybridomas were screened by an antibody-sandwich enzyme-linked immunosorbent assay (ELISA) and cloned by limiting dilution. The isotype of the MAbs were determined using an isotyping kit for monoclonal antibodies (Serotec).

The antibody-sandwich ELISA was performed as described below. Microtiter plates were coated with anti-MEV rabbit serum [4] in 0.05 M sodium carbonate buffer (pH 9.6) overnight at 4°C and then blocked with 5% fetal calf serum (FCS) in phosphate-buffered saline (PBS). Fifty microliters of virus-infected culture supernatants were added to the wells and incubated for 1 hr at room temperature (r.t). The supernatants of hybridomas were added to the wells and the mixtures were incubated for 1 hr at r.t. After washing the plate with PBS containing 0.2% Tween 20, a horseradish peroxidase-conjugated donkey serum anti-mouse immunoglobulins (1:1,000 dilution) (Amersham) was added and incubated for 1 hr. Antigen-antibody complexes were visualized with a substrate solution (0.1 mg/m*l* 2,2'-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid], 0.02% H_2O_2, in 0.05 M citrate buffer [pH 4.0]) and optical density at 405 nm was measured by a microplate reader (Bio-Rad model 2550).

Epitope of the FPLV/MEV-specific MAb was determined by using a hemagglutination inhibition (HI) test [13] and antibody-sandwich ELISA with CPV/MEV chimeric viruses. The genomic organization of the CPV/MEV chimeric viruses have been described [5], except for v2, v7 and v8. The recombinant virus v2 possessed a single mutation at nucleotides (nt) 3103 in the MEV background (vMEV→v2:AAA→AA<u>C</u>, underline indicates nucleotide targeted for site-directed mutagenesis), in which the amino acid (aa) 93-Lys in MEV VP2 was changed to Asn in v2. The

recombinant virus v7 possessed a single mutation at nt 3132 (vMEV→v7:GTA→GCA), in which aa 103-Val in MEV VP2 was changed to Ala in v7. The recombinant virus v8 possessed a single mutation at nt 3791 (vMEV→v8:GAC→AAC), in which aa 323-Asp in MEV VP2 was changed to Asn in v8. Strategies and primers used for the site-directed mutagenesis have been described previously [5]. For immunoblot analysis, CsCl-isopycnic centrifugation-purified MEV-Abashiri strain was lysed with a sample buffer (2% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue, in 0.1 M Tris-HCl, pH 6.8), electrophoresed on a 10% SDS-polyacrylamide gel, and transferred onto Immobilon™ transfer membranes (Millipore). The blots were incubated with MAbs and the second antibody, and the antigen-antibody complexes were detected using the ECL Western blot detection system (Amersham).

Fifteen hybridomas secreting MAb against MEV-Abashiri were established. Table 1 shows the properties of the MAbs. Fourteen of them reacted with CPV-Y1, FPLV-Obihiro, and MEV-Abashiri, indicating that they recognized the common epitope of the feline parvovirus subgroup. One MAb, designated as P2–215, reacted with FPLV-Obihiro and MEV-Abashiri, but not with CPV-Y1. Further analysis of the cross-reactivity of the MAb P2–215 using several field isolates revealed that the MAb P2–215 reacted only with FPLV/MEV isolates but not with CPV isolates, whereas the other MAbs used for the examination reacted with all the isolates (Table 2). Thus, we determined that MAb P2–215 recognized the FPLV/MEV-specific epitope. MAbs P1–72, P1–96, P1–208, P1–260, P1–269, and P2–168 reacted with virus capsid antigens, VP1 and VP2, in immunoblot analysis (Fig. 1), while all of them were negative in the HI test (Table 1); the remaining MAbs did not react with the capsid antigens in immunoblot analysis. The MAbs P1–222, P1–224, P2–29, P2–215, P2–284, and X1–251 showed virus neutralizing activity (data not shown).

The cross-reactivity analysis revealed that MAb P2–215 was specific to FPLV/MEV (Table 2). To determine the epitope of MAb P2–215, the reactivity of MAb P2–215 with various CPV/MEV chimeric viruses was analyzed using antibody-sandwich ELISA and HI test (Fig. 2). The MAb P2–215 reacted with v3P and vV5: the ratio of P2–215/X1–251 was nearly 1 and P2–215 inhibited the HA activity of v3P and vV5. This means that the regions from the initiation sites of VP1 and VP2 to the *Pst* I site (VP1: map units [m.u.] 45 to 60; VP2: m.u. 55 to 60), and from the *Eco* RV site to the end of the VP1 and VP2 genes (m.u. 79 to 89) were not part of the MAb P2–215 epitope. The MAb did not react with vP5 and vPB, indicating that the genomic region from the *Pst* I to the *Bgl* II sites (m.u. 60 to 66) encodes amino acids that constitute the epitope for MAb P2–215. Between FPLV/MEV and CPV, two FPLV/MEV-specific amino acids were present within this region (aa 93 and 103 in VP2 [5, 10]). The MAb P2–215 reacted with the recombinant virus v7 but not with v2, indicating that one FPLV/MEV-specific amino acid residue, aa 93-Lys, constitutes the epitope for MAb P2–215. This was also confirmed through the reactivities of MAb P2–215 against the recombinant viruses v28, v8, and v78. MAb P2–215

Table 1. Properties of MAbs against MEV-Abashiri

MAb[b]	ELISA[a]			HI	Immuno-blot	Isotype
	CPV	FPLV	MEV			
P1–68	2560[c]	2560	2560	8	–	IgG2a
P1–72	40	40	40	<8	+	IgG1
P1–96	40	10	10	<8	+	IgG1
P1–105	2560	640	640	<8	–	IgG1
P1–208	40	40	40	<8	+	IgG3
P1–222	10240	10240	10240	128	–	IgG2a
P1–224	10240	10240	10240	16	–	IgG2a
P1-260	640	40	160	<8	+	IgG1
P1–269	40	160	40	<8	+	IgG1
P2–29	10240	10240	10240	64	–	IgG2a
P2–168	640	640	640	<8	+	IgG2b
P2–215	<10	10240	10240	512	–	IgG2a
P2–284	10240	2560	2560	64	–	IgG1
X1–222	10240	640	2560	16	–	IgG2a
X1–251	10240	10240	10240	1024	–	IgG1

a) Viruses used were: CPV, CPV-Y1; FPLV, FPLV-Obihiro; MEV, MEV-Abashiri.
b) Prefix "P" means hybridomas derived from P3-X63.Ag.U1, while "X" means those from P3-X63.Ag.8.653.
c) The titers are given as reciprocals of the last dilution of culture supernatant giving a positive signal.

Table 2. Cross-reactivities of MAb P2–215[a]

MAb[b]	CPV			FPLV			MEV	
	Cp49[c] (1979)[d]	Y1 (1982)	Kushiro (1982)	TU1 (1973)	Obihiro (1974)	483 (1990)	Abashiri (1978)	M1 (1988)
P2–215	–	–	–	+	+	+	+	+
P1–222	+	+	+	+	+	+	+	+
P2–284	+	+	+	+	+	+	+	+
X1–251	+	+	+	+	+	+	+	+

a) Determined by antibody-sandwich ELISA.
b) Culture supernatants were used at a dilution of 1:20.
c) Viruses used were listed elsewhere [5, 6], except for M1 [7].
d) Year of isolation.

did not react with the recombinant viruses with a CPV-type aa 93 in VP2.

Between CPV and FPLV/MEV, only six amino acid differences occur consistently in VP2 [10]. The three dimensional structures of both CPV and FPLV have been determined [1, 18], and those show the location of the FPLV/MEV-specific amino acid residues on the three dimensional structure of the VP2 [1]. In this study, the reactivity of MAb P2–215 against the recombinant viruses revealed that this MAb recognizes the conformational epitope containing aa 93-Lys in VP2 of FPLV/MEV. The aa 93 is a surface residue on the three-hold spike of the virion, and constitutes one neutralizing epitope, the antigenic site A, together with aa 222, 224, and 426 [1, 15]. The MAb P2–215 has virus-neutralizing activity but did not react with the capsid antigens in immunoblot analysis, indicating that MAb P2–215 recognizes an epitope similar to the antigenic site A, which is folded as a FPLV/MEV-specific structure.

With respect to FPLV/MEV-specific MAb, two rat MAbs (MAb G and H) have been characterized which recognize FPLV to higher titers than CPV [2, 9], however, the epitope for MAb P2–215 obviously differed from those for MAbs G and H. MAbs G and H reacted with the recombinant virus vBI322 (corresponds to v2 here), which had the aa 93-Asn in the FPLV genetic background; and indeed, the epitopes recognized by MAb G and H have not been clearly defined [2]. In contrast, the substitution at aa 93-Lys to Asn drastically affected the epitope for MAb P2–215 (Fig. 2). This clearly demonstrated that the FPLV/MEV-specific epitope associated with a single sequence at aa 93-Lys was actually present, as was the CPV-specific epitope associated with aa 93-Asn [12]. Definition of the epitope recognized by MAb P2–215 makes selection and analyses of monoclonal variants of MAb P2–215 possible.

The virus isolates have been named according to the host from which they were isolated. Recently, however, CPV-2a and/or 2b were isolated from domestic cats manifesting

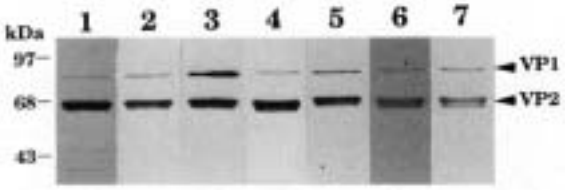
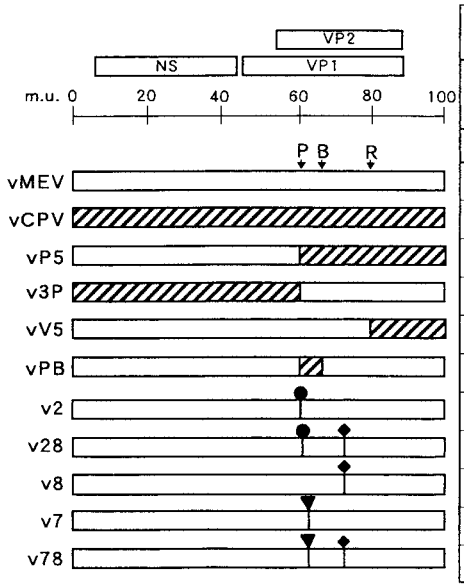


Fig. 1. Reactivity of MAbs in immunoblot analysis. Lanes 1, P1–72; 2, P1–96; 3, P1–208; 4, P1–260; 5, P1–269; 6, P2–168; 7, anti-MEV rabbit serum.

a



b

FPLV/MEV vs. CPV-specific amino acid in VP2						Reactivity of MAb P2-215			
P ↓			B ↓	R ↓ (m.u.)		ELISA			HI
						A405nm		Ratio	
80	93	103	323	564	568	P2-215	X1-251	P2-215/X1-251	
K	K	V	D	N	A	0.855	0.868	0.99	1024
R	N	A	N	S	N	0.018	0.843	0.02	<4
K	N	A	N	S	N	0.007	0.694	0.01	<4
R	K	V	D	N	A	1.243	1.376	0.90	512
K	K	V	D	S	N	0.762	0.697	1.09	1024
K	N	A	D	N	A	0.012	1.080	0.01	<4
K	N	V	D	N	A	0.017	0.378	0.04	<4
K	N	V	N	N	A	0.015	0.284	0.05	<4
K	K	V	N	N	A	0.311	0.299	1.04	1024
K	K	A	D	N	A	0.383	0.380	1.01	1024
K	K	A	N	N	A	0.278	0.287	0.97	1024

Fig. 2. Epitope analysis of MAb P2–215. (a) Genetic organization of the recombinant viruses. The three boxes on top indicate the NS, VP1 and VP2 open reading frames. Arrow with capital letter indicates restriction site used for construction of the CPV/MEV chimeric plasmid: P, *Pst* I (m.u. 60); B, *Bgl* II (m.u. 66); R, *Eco* RV (m.u. 79). Genetic region of MEV indicates open box, while that of CPV indicates hatched box. Single mutations at nt 3103, 3132, and 3791 are indicated as a vertical bar with filled circle, a reverse triangle, and a diamond, respectively. (b) Amino acid profile of the recombinant viruses and reactivity of MAb P2–215. Although there are 11 amino acid differences in VP2 between MEV-Abashiri and CPV-Y1 that are used for production of recombinant viruses [5], only the FPLV/MEV versus CPV-specific amino acids are shown in the figure. CPV-specific amino acids are highlighted. A 1:10 dilution of culture supernatants of MAbs P2–215 (FPLV/MEV-specific) and X1–251 (CPV and FPLV/MEV-common) were used for ELISA, and the absorbance at 405 nm is shown. Cut off value of the ELISA was 0.049. HI titers are given as the reciprocal of the last dilution of culture supernatant that inhibited 8 HA units.

symptoms of feline panleukopenia [8, 17]. Experimental infection of CPV-2a and 2b in cats demonstrated that CPV-2a and 2b replicate in the ileum, thymus, and mesenteric lymph node of cats [17]. For diagnosis, our FPLV-specific MAb and PCR-restriction fragment length polymorphisms (RFLP) analysis targeted to the nucleotides differences between CPV and FPLV/MEV [6], will specifically identify virus strains.

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