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Original Article

Characterization of a nonhemagglutinating mutant of mink enteritis virus in China

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Abstract: Parvoviruses are small eukaryotic DNA viruses that infect a variety of animal species, including humans. Canine parvovirus type-2, feline panleukopenia virus and mink enteritis virus are all host-range variants of the carnivore parvovirus subgroup. These viruses could hemagglutinate (HA) swine or rhesus monkey erythrocytes in buffered saline solutions at pHs between 6.0 and 6.8. Here we report a nonhemagglutinating mutant of MEV and the amino acid differences compared with other MEV, FPV and CPV strains. Further studies and investigations on MEV are needed to clarify whether this mutant is fixed in the MEV population as a consequence of the mechanisms of antigenic escape or further adaptation to broader host range. It turns crucial to have a better understanding of the epidemiology and evolution of parvovirus.

Keywords: MEV, hemagglutination, amino acids

Introduction

Parvoviruses (family *Parvoviridae*) are small eukaryotic DNA viruses that infect a variety of animal species, including humans. Canine parvovirus (CPV), feline panleukopenia virus (FPV), and mink enteritis virus (MEV) are all host-range variants of the carnivore parvovirus subgroup [1, 2]. Strikingly, although these single-stranded viruses have a DNA genome and use cellular replication machinery, their rate of nucleotide substitution and the underlying rate of mutation is same as that of RNA viruses [3]. CPV emerged in the mid-1970s as a new pathogen of dogs and has since become endemic in the global dog population. Despite widespread vaccination, CPV has remained a persistent disease of dogs. The new genetic and antigenic variants have arisen and extended host range. The CPV represents a rare and important model of disease emergence through cross-species transmission [4]. It is known that several amino acids in the capsid protein of CPV coordinately determined the canine host range, specific antigenic and HA properties [5]. MEV is genetically and antigenically closely related to CPV. These viruses could hemagglutinate (HA) swine or rhesus monkey erythrocytes in buffered saline solutions at pHs between 6.0 and 6.8 [6]. The first nonhemagglutinating MEV was identified and designated MEV-S in 1984 Sweden [7]. However, the nature of hemagglutination (HA) by parvovirus is not well understood. Differences of amino acids in the capsid protein sequences between HA and Non-HA MEV strains have not been reported previously. Here we report a nonhemagglutinating mutant of MEV and the amino acid differences compared with other MEV strains.

In 2009, an infectious enteritis outbreak occurred, which affected more than 5,000 minks at a breeding mink farm in Shandong province of China. Nearly all minks showed clinical and pathological signs of enteritis and the mortality was estimated at 50%. We successfully isolated the pathogenic agent from the fecal samples of infected minks by repeated passages in Crandell Rees feline kidney (CRFK) cells. It was designated as MEV-SDNH. Virus isolation (VI) was conducted as previously described [8]. Two days after incubation, the inoculated cells were tested by direct immunofluoresence (IF) assay using anti-CPV monoclo-

Table 1. Changes of amino acid in capsid protein VP2 for MEV, FPV and CPV strains

Strains	Amino acid position				GenBamk accession no.
	236	300	413	426	
MEV-Abashiri	Thr	Ala	Asp	Asn	D00765
MEV-d	-	-	-	-	U22190
MEV-e	-	-	-	-	U22191
MEV-Antigenic type 1	-	-	-	-	M23999
MEV-Antigenic type 2	-	Val	-	-	M24001
MEV-DL	-	Val	Gly	-	HM015824
MEV-LN10	-	Val	Asp	-	HQ694567
MEV-SDNH	Ser	Ile	Asn	Lys	JX535284
FPV	Thr	Ala	Asp	Asn	M38246
CPV-2	-	Ala	-	-	M38245
CPV-2a	-	Gly	-	-	M24003
CPV-2b	-	-	-	Asp	M74849
CPV-2c	-	-	-	Glu	FJ005204

In order to simplify the presentation of results, the amino acid sequences identical with the above positions are represented by dashes.

nal antibody conjugated with fluorescein isothiocyanate [9]. The titer of MEV-SDNH was $10^{3.2}TCID_{50}$/ml determined by IF staining. Six minks were challenged subcutaneously with MEV-SDNH and monitored for clinical signs of mink enteritis and virus shedding. All injected animals displayed the typical signs of infection with pathogenic MEV and the virus could be re-isolated from fecal material. While the six controls remained free of enteric signs and did not shed virus in the feces.

Both the isolate MEV-SDNH strain and the original feces from infected minks involved in the outbreak farm were tested for hemagglutinating activity. Hemagglutination (HA) assays were performed as previously described [10]. The isolate and the original fecal suspension did not agglutinate porcine or rhesus monkey erythrocytes at temperature of 4°C and pH 6.4. The positive control virus (MEV-LN10 strain, GenBank accession No. HQ694567) was shown to agglutinate the erythrocytes under the same conditions. The nonstructural and capsid protein genes of MEV-SDNH were amplified and sequenced (GenBank accession no. JX535284). The capsid protein VP2 amino acid sequences were compared among MEV, FPV, CPV-2, CPV-2a, CPV-2b and CPV-2c strains. We found 4 substitutions in amino acid sequence, including Thr→Ser at residue 236, Val/Ala→Ile at residue 300, Asp/Gly→Asn at residue 413 and Asn→Lys at residue 426, between the MEV-SDNH and non-HA MEV strains (**Table 1**). These changes may be relevant for the loss of HA ability.

A non-HA mutant of CPV was a single Arg→Lys difference of VP2 amino acid residue 377. The pH dependence of binding of the closely related FPV was shown to result from a decreased binding in buffers with pH values of 6.8 or greater. The VP2 residues responsible for that difference have been shown to be 323 and 375 [11]. But the non-HA CPV was derived during in vitro passage in the NLFK cell line and presumably represented a mutant better adapted for growth in cells [6]. The natural loss of HA ability of MEV did not seem to influence the infectivity in vitro or vivo. It should reflectsome changes in the viral erythrocyte binding site. The substitutions which affect that site have not been defined. It was indicated that carnivore parvovirus is subject to purifying selection, with adaptive evolution restricted to specific residues within capsid VP2 [12]. The VP2 amino acid differences of CPV at some positions defined the new antigenic types CPV-2a, CPV-2b and CPV-2c. The CPV-2c is characterized by an alternate mutation at VP2 residue 426 points to a special role of this residue in infection or immune evasion and that codon is shown to be under positive selection in the viral genome [4]. Residue 426 is situated in epitope A, over the threefold spike of the capsid, and a role of antigenic escape has been assigned to the same residue of the VP2 of the parvovirus minute virus of mice [12]. Amino acid 426 is conserved in FPV, CPV-2 and CPV-2a viruses but is different in CPV-2b/2c viruses. In contrast to other hemagglutinating MEV strains reported, the MEV-SDNH coordinately differed at residue 426 in capsid VP2. It is possibly a new antigenic variant of MEV that has not been reported previously (**Table 1**).

A nonhemagglutinating mutant of mink enteritis virus was derived and identified by VI, IF, HA, and animal infection tests. There were 4 amino acid changes in the capsid protein VP2 compared to HA MEV strains. It was noteworthy that the mutation at the residues position 426 led to Asn→Lys replacement.

The biological consequences of these mutations are not clear, but new variants were found

to acquire definite advantages over the original CPV. Changes at residue 426 have modified the antigenic profile of CPV since it is located in the major antigenic region over the three-fold spike of the CPV capsid, and molecular epidemiological studies have demonstrated that complicated selection dynamics act on this residue [13, 14]. However CPV-2 was unable to replicate in cats, the new variants of canine parvovirus, CPV-2a and CPV-2b/2c have also penetrated the feline host-range and they were able to infect and replicate in cats [15]. Residue 426 has undergone two mutations since the emergence of CPV, and is the only coding changes capable of distinguishing the CPV-2a strains from CPV-2b/2c strains [16]. The adaptation of CPV was likely dependent on a high rate of mutation and the positive selection of mutations in the major capsid gene [3]. Mutations involving residue 426 have not been reported previously for MEV. Analogously to CPV, it may be hypothesized that this change may affect the antigenic profile of MEV and confer an evolutionary advantage to the virus. Further studies and investigations on MEV are needed to clarify whether this mutant is fixed in the MEV population as a consequence of the mechanisms of antigenic escape or further adaptation to broader host range. It turns crucial to have a better understanding of the epidemiology and evolution of parvovirus.

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Disclosure of conflict of interest

None.

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