Short communication

Identification of a novel Aleutian mink disease virus B-cell epitope using a monoclonal antibody against VP2 protein

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A B S T R A C T

Aleutian mink disease virus (AMDV) is a parovirus that causes an immune complex-mediated disease in minks. Capsid protein VP2 is a major structural viral protein and can be used to diagnose AMDV. In this study, a specific monoclonal antibody, 1M13, was produced against the AMDV VP2 protein (amino acids 291–502). A linear VP2-protein epitope was identified by subjecting a series of partially overlapping synthesized peptides to be enzyme-linked immunosorbent assay (ELISA) analysis. The results indicated that SGLQSQSTFLQVNLK(306) was the minimal linear epitope that could be recognized by mAb 1M13. ELISA assays revealed that mink anti-AMDV sera could also recognize the minimal linear epitope. Sequence alignments demonstrated that the linear epitope is highly conserved among AMDV strains except SGLH and is less conserved among Raccoon dog amdovirus, Gray fox amdovirus, Red fox amdovirus, Bat parvovirus and Mink enteritis parvovirus. Taken together, the generation of this VP2-specific mAb with a defined linear peptide epitope may have potential applications in the development of suitable diagnostic techniques for AMDV.

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Aleutian mink disease (AMD), caused by the Aleutian mink disease virus (AMDV), is the most commercially important infectious disease affecting farmed mink (Neovison vison) worldwide and causes considerable economic losses to mink farmers through both reduced reproductive output and decreased fur value(Alexandersen, 1990; Gottschalck et al., 1991; Nituch et al., 2011). AMDV is a member of the genus Amovirus, subfamily Parovirinae and family Paroviridae. It contains a 4.8-kb single-stranded DNA genome that codes for three nonstructural proteins (NS1, NS2, and NS3) and two structural proteins (VP1 and VP2) (Bloom et al., 1994).

VP2 proteins are the major immunogenic antigens of ADV and are closely related to viral tropism, pathogenicity, and host selection. Recombinant AMDV VP2 proteins have been expressed and are shown to be antigenic and able to form virus-like particles (VLPs) (Clemens et al., 1992). Furthermore, many diagnostic applications have been described (Chen et al., 2015; Knuuttila et al., 2009) that are based on AMDV VLPs or a truncated VP2 protein. However, there is a great deal that remains unknown about the structure and function of the AMDV VP2 protein. In this study, we prepared a monoclonal antibody (mAb) against the AMDV VP2 protein and defined the linear epitopes recognized by the mAb. We anticipate that these reagents and results will provide a foundation for the development of AMDV diagnostic technologies and facilitate studies in the structure and function of the AMDV VP2 protein.

The ADV-G (GenBank accession no. M20036) strain was lab-adapted and propagated on CRFK cells. The truncated ADV VP2 gene coding sequence was amplified using sequences as follows: (5′-GGCGATGCATGGAATGGCCT3′) and (5′-GCTCGACTGACTGTCCT3′). The DNA fragment encoding amino acids 291–502 of the VP2 protein was introduced into the KpnI/Xhol sites of the E. coli expression vector pET30a (Qia- gen, Hilden, Germany) to produce a recombinant protein with a histidine-tag. The recombinant protein was induced by adding isopropyl-β-thiogalactopyranoside (IPTG). The His-tagged protein was purified by Ni-Agarose Resin for 6 × His-Tagged protein (CWBio, China). The production of recombinant protein was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and used as the antigen for generating MAbs. The purified protein was detected as a single band with the expected molecular weight of approximately 32 kDa (Fig. 1a) and used as the antigen to generate MAbs.

We immunized BALB/c mice with the purified recombinant truncated ADV-G VP2 (aa 291–502) to generate hybridoma lines

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Fig. 1. Characterization of the recombinant truncated VP2 protein and the monoclonal antibody 1M13. (a) SDS-PAGE analysis of the recombinant truncated VP2 protein after His Resin purification. M, molecular weight marker; lane 1, blank; lane 2–10, purified recombinant truncated VP2 protein with different iminazole concentrations: 10 mM, 20 mM, 40 mM, 60 mM, 80 mM, 100 mM, 120 mM, 160 mM, 180 mM, and 200 mM. (b) Characterization of AMDV VP2-reactive mAb 1M13 using western blotting. M: Prestained Protein Ladder; lane 1, lysate of Mink enteritis parvovirus-infected CRFK cells; lane 2, lysate of AMDV-infected CRFK cells; lane 3, lysate of uninfected CRFK cells. (c) CRFK cells were infected with ADV-G and used to evaluate mAb reactivity by IFA. (d) uninfected CRFK cells represent the negative control.

Secreting antibodies against the AMDV VP2 protein. Hybridoma cell supernatants were screened by indirect enzyme-linked immunosorbent assay (ELISA), and selected hybridoma lines were subcloned by limiting dilutions. One hybridoma line that produced a stable antibody against AMDV VP2 protein was obtained and named 1M13.

WB analysis revealed that the mAb produced by the hybridoma line reacted with the native VP2 protein derived from ADV-infected CRFK cells, and it did not recognize uninfected CRFK cell lysates and MEV (Fig. 1b). The sizes of the ADV VP2 proteins recognized by the 1M13 mAb are approximately 85 kD and 75 kD, respectively. These results are consistent with a previous ADV G epitope study (Kierek-Jaszczuk et al., 1986). In addition, the mAb was able to react with CRFK cells infected with ADV-G by IFA (Fig. 1c) but did not bind appreciably to uninfected CRFK cells (Fig. 1d). Furthermore, the mAb consisted of an IgG1 heavy chain and kappa light chain, as determined by the Mouse Monoclonal-Ab-ID kit.

We next sought to define the linear epitope within the AMDV VP2 protein (aa 291–502) recognized by mAb 1M13. Peptide scanning technology was used to prepare a series of 36 overlapping synthetic peptides. Each 15 aa peptide overlapped the previous peptide sequence by 10 aa residues. An indirect ELISA was used to screen the series of synthetically produced truncated VP2-derived peptides. The linear epitope within the AMDV
VP2 protein was identified (Fig. 2a). mAb 1M13 recognized synthetic peptide-23, which contained the VP2-derived sequence \(386^{	ext{HLQQNFSTRYIYD}}398\). To further refine our definition of the epitopes recognized by the 1M13 mAb, we tested peptides with progressive deletions of the C- and N-termini of the motifs identified in the initial peptide screen. A peptide lacking the C-terminal \(386^{	ext{HL}}387\) residue of this core peptide sequence displayed a strong reduction in 1M13 binding, where the OD450 value decreased from 0.53 to 0.12. When residues at the N-terminus were progressively deleted, little reduction in 1M3 binding was noted until the residue \(386^{	ext{HL}}387\) was deleted, where the OD450 value decreased from 1.21 to 0.53. Therefore, we demonstrated that \(386^{	ext{HL}}387\) and \(386^{	ext{D}}\) are critical components of the linear epitope. So, the minimal peptide epitope recognized by mAb 1M13 was \(386^{	ext{HL}}387\) (Fig. 2b).

To investigate whether the epitopes could be detected by AMDV-positive mink serum, the BSA-fused synthetic peptides containing \(386^{	ext{HLQQNFSTRYIYD}}398\) were used to react with AMDV-positive mink serum by ELISA. The result showed that AMDV-positive mink serum recognized the identified B cell epitope but did not react with AMDV-negative mink serum (Fig. 3a). Amino acids were progressively deleted from the N- or C-terminus of the peptide sequence, and their reaction with AMDV-positive mink serum gradually declined. This demonstrates that the identified B cell epitope is targeted by the mink immune response in the context of AMDV infection.

We assessed the level of conservation of the identified linear epitope among representative viruses of AMDV as well as relative members of AMDV, including Raccoon dog amdogivirus (Shao et al., 2014), Gray fox amdogivirus, Red fox amdogivirus (Li et al., 2011), Bat parvovirus and MEV. The alignment results showed that the defined epitope \(386^{	ext{HLQQNFSTRYIYD}}398\) was completely conserved among all AMDV strains except \(386^{	ext{HL}}387\), where a Y-F substitution at position 397 was noted in Raccoon dog amdogivirus, a Y-VF substitution at position 396–397 was noted in Gray fox amdogivirus, and a Y-VW substitution at position 395–397 was noted in Red fox amdogivirus (Fig. 3b). In contrast, the AMDV VP2 epitope was not completely conserved in Bat parvovirus \(386^{	ext{HFQNYRTYD}}388\) and Mink enteritis parvovirus \(386^{	ext{GDPRYAFGR}}388\).

With the trends of global fur product trade and the expansion of development in the mink-breeding industry, AMDV disease has raised global attention with its extensive spreading, which includes Europe, North America, and China. Since the introduction and propagation of mink in the late 1950s for the fur industry in China, the mink population has become the world’s largest number at present. Counter immunoelectrophoresis (CIEP) has been widely used for routine detection of AMDV antibodies (Dam-Tuxen et al., 2014). However, the CIEP analysis is labor intensive and complicated, which is not suited for large-scale detecting.

To date, the diagnosis and prevention of AMDV are more difficult and complicated. It is crucial to diagnose AMDV quickly and efficiently. Some ELISA assays based on the expressed ADMV VP2 protein has been developed (Chen et al., 2015; Dam-Tuxen et al., 2014; Knuuttila et al., 2014), but the detailed sequence of linear B-cell epitopes of the AMDV VP2 protein remains undefined.

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**Fig. 2.** Identification of minimal linear epitopes recognized by mAb 1M13. a. 1M13 was screened by indirect ELISA against a panel of 36 overlapping peptides derived from the AMDV VP2 amino acid 291–502 sequence. A CDV N-reactive antibody was used as a negative control. The error bars display the standard deviation of three experimental repeats. b. 1M13 was screened against a series of truncated peptides based on the result of the initial screen shown in panel a. A CDV N-reactive antibody was used as a negative control. c. The name and sequence of the peptides used are included in the table.

**Fig. 3.** Analysis of the epitope sequence of HLQQNFSTRYIYD. a. Indirect ELISA analysis of the interactions between the artificial progressively peptides BSA-HLQQNFSTRYIYD and the AMDV-positive/negative mink serum. BSA was used as a negative control. B. Alignment of the identified minimal linear peptide epitope with corresponding sequences of AMDV strains, Raccoon dog amdogivirus, Gray fox amdogivirus, Red fox amdogivirus, Bat parvovirus and Mink enteritis parvovirus.

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A detailed analysis of the antibody epitopes within the VP2 protein would further our understanding of AMDV immunity and the development of epitope-based diagnostic tools. Moreover, this is the first report of an epitope (386HLQNFSTRYIYD398) in the AMDV VP2 protein, which can be potentially valuable for further AMDV research.

A previous study performed epitope mapping of AMDV using poly-antibodies (Costello et al., 1999), but the detailed sequence of linear B-cell epitopes of the AMDV VP2 protein remains undefined. Interestingly, this analysis was consistent with the previous result, which demonstrated that a dominant AMDV VP2 linear epitope in protein RP3b was located between amino acids 380–518 of the VP2 protein. In summary, we generated an AMDV VP2 reactive mAb and defined a novel linear B-cell epitope. The generation of this VP2-specific mAb with a defined linear peptide epitope may serve as a foundation for studies aiming to elucidate the role of VP2 protein–protein interactions on virus replication and may be better for developing a new AMDV diagnostic system.

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References


