

Monoclonal Antibody Against M Protein of Spring Viremia of Carp Virus

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M protein of spring viremia of carp virus (SVCV) was expressed in *Escherichia coli* and then used to immunize BALB/c mice. One monoclonal antibody (5A1) against M protein was generated by fusion of mouse myeloma cell line SP2/0 and spleen lymphocytes from immunized mice. The characterizations of this MAb were confirmed by ELISA (enzyme linked immunosorbent assay), IFA (immunofluorescent assay), and Western blot analysis. All results indicate that MAb 5A1 was specific to SVCV M protein.

Introduction

SPRING VIREMIA OF CARP VIRUS (SVCV) is the pathogen of infectious hemorrhagic swimbladder inflammation in common carp, which could cause high mortality in juvenile carp (30–70%). In 2006, it was listed as noticeable in the International Aquatic Animal Health Code of the Office International des Epizooties (OIE).⁽¹⁾ The initial outbreak of this disease was in European countries. However, recently it has been reported in the United States, Middle East, China, Canada, and Brazil.⁽²⁾

SVCV, one of the rhabdoviruses, is a member of Rhabdoviridae, genus *Vesiculovirus*.⁽³⁾ The genome of the virus is situated in an elongated nucleocapsid and surrounded by a lipoprotein layer with glycoprotein projections. It encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and viral RNA-dependent RNA polymerase (L).^(4,5) The M protein is composed of 223 amino acids and has about 31.6% homology with the M protein of vesicular stomatitis virus (VSV).⁽⁶⁾ Previous studies reported that the M protein could cause apoptosis and play an important role during the cytopathic effect.^(7,8) Furthermore, M protein could also inhibit the transcription of host cells.⁽⁹⁾

In this study, we generated an MAb against the M protein of SVCV. This MAb can be a useful tool for the development of detection methods for SVCV and studying the function of M protein.

Materials and Methods

Cloning, expression, and purification of M protein

M gene was amplified from SVCV-infected epithelioma papulosum cyprini (EPC) cells by a one-step RT-PCR. The

primers of this gene are listed in Table 1. The M gene fragment was then inserted into the bacterial expression vector pGEX-KG and named the recombinant plasmid KG-SVCV/M. KG-SVCV/M and control plasmid pKG were transformed into *Escherichia coli* BL21 cells and induced by isopropyl- β -thio-galactopyranoside (IPTG) at 37°C. Four hours later, bacterium was collected by centrifugation. Then, the bacterial pellet was resuspended and sonicated until clear lysate was obtained. The M protein was purified by dialysis and then stored at –80°C.⁽¹¹⁾

Production of monoclonal antibody

Female BALB/c mice, about 4 weeks old, were immunized intramuscularly with 60 μ L of a 1:2 purified M protein (30 μ g per mouse) and Quickantibody adjuvant. After 2 weeks, the mice were immunized again with the same mixture. Two weeks later, before fusion, the mice were boosted with 60 μ g of M protein, and 3 days later, mice splenocytes were harvested and fused with SP2/0 using 50% polyethylene glycol (PEG4000). The fused cells were cultured in HAT medium with fetal bovine serum. Ten days later, the aminopterin was omitted and fused cells were cultured in HT medium. The supernatants of hybridoma culture were screened by ELISA. Then, the positive hybridoma lines were chosen to subclone three times using limiting dilution method. The stable hybridoma clones were injected into BALB/c mice intraperitoneally.⁽¹²⁾ Subsequently, seroperitoneum was collected from the immunized mice and the MAb was purified by the antibody purification kit according to the manufacturer's instructions.

Identification of MAb subtype

Subtype identification kit (Pierce Rapid ELISA Mouse MAb Isotyping Kit, Thermo Scientific, Boston, MA) was

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TABLE 1. PRIMER SEQUENCES

Gene	Primer orientation	Primer sequence (5'-3')
KG-SVCV/M	Forward	AAAGGATCCATGTCTACTCTAAGAAAGCTC
	Reverse	AAACTCGAGATCTCCCATGAACAGGGAA
KG-SVCV/Ma	Forward	AAAGGATCCATGTCTACTCTAAGAAAGCT
	Reverse	AAACTCGAGTTACGACATCGTTTTTAAAGGGTGT
KG-SVCV/Mb	Forward	AAAGGATCCATGCCCTTAAAAACGATGTCGGA
	Reverse	AAACTCGAGTTAACTCCCTTGCACCTTGTTAGA
KG-SVCV/Mc	Forward	AAAGGATCCATGAACAAGGTGCAAGGGAGTTGT
	Reverse	AAACTCGAGTTACTGACACTCTTTCAACAAATTG
KG-SVCV/Md	Forward	AAAGGATCCATGTTGTTGAAAGAGTGTCCAGGTAG
	Reverse	AAACTCGAGTTAATCTCCCATGAACAGGGAA

used to identify the subtype of the MAb according to the manufacturer's instructions.

Immunofluorescence assay

EPC cells were cultured in a 24-well plate (Costar Corning, Corning, NY) and infected with SVCV at one multiplicity of infection (MOI). Twenty-four hours post-infection, EPC cells were fixed with absolute methanol and processed for IFA using MAb 5A1, followed by the secondary antibody fluorescein isocyanate-conjugated goat anti-mouse IgG. Fluorescent images were examined under a fluorescent microscope.

Western blot analysis

Western blot assay was used to analyze the specificity of MAb 5A1. EPC cells were infected by SVCV for 36 h; then cells were lysed to collect the lysates. Samples were separated by 12% SDS-PAGE and transferred to the nitrocellulose membrane. The membrane was blocked overnight with 1% bovine serum albumin (BSA) in TBST buffer at 4°C, then incubated with MAb 5A1 (diluted 1:500) at 37°C for 1 h. After washing the membrane three times with TBST buffer, the membrane was incubated with the secondary antibody (horseradish peroxidase [HRP]-conjugated goat anti-mouse IgG; diluted 1:1000) at 37°C for 1 h. After washing three

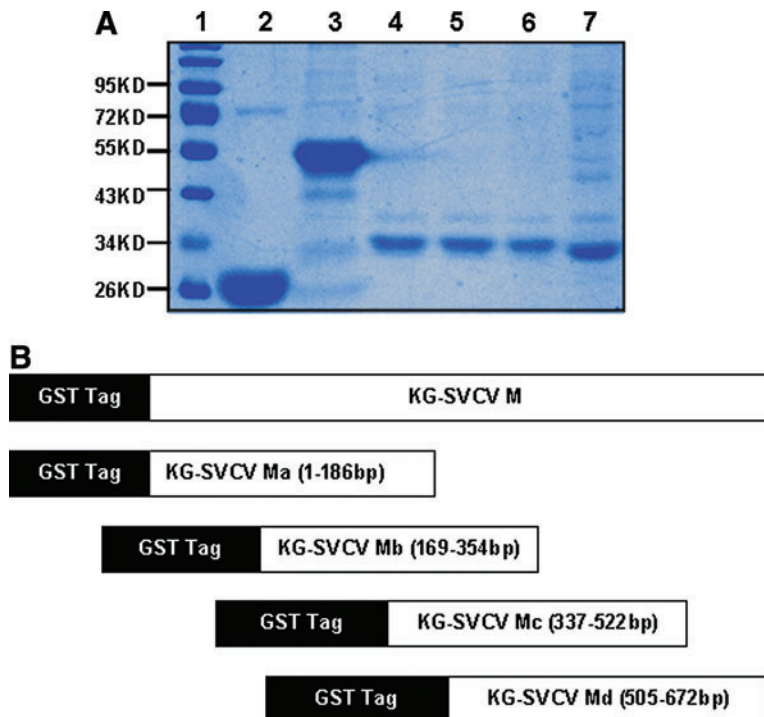


FIG. 1. SDS-PAGE analysis of recombinant proteins KG-SVCV/M, KG-SVCV/Ma (1-186bp), KG-SVCV/Mb (169-354bp), KG-SVCV/Mc (337-522bp), KG-SVCV/Md (505-672bp). (A) Lane 1, protein marker; lane 2, bacilli precipitation of pGEX-KG; lane 3, bacilli precipitation of KG-SVCV/M; lane 4, bacilli precipitation of KG-SVCV/Ma (1-186bp); lane 5, bacilli precipitation of KG-SVCV/Mb (169-354bp); lane 6, bacilli precipitation of KG-SVCV/Mc (337-522bp); lane 7, bacilli precipitation of KG-SVCV/Md (505-672bp). (B) Construction of recombinant plasmids expressing full length or truncated forms of M protein.

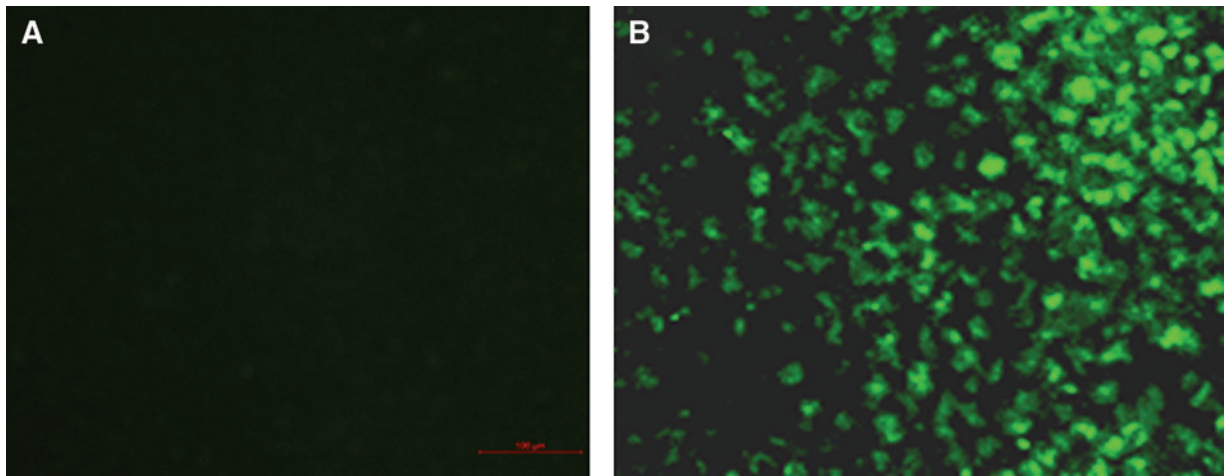


FIG. 2. Immunofluorescence staining (IFA) to evaluate MAb 5A1. (A) EPC cells were infected with SVCV. 24 h post-infection, fluorescent images were examined with a fluorescent microscope using MAb 5A1 as primary antibody and Alexa Fluor 488 goat anti-mouse IgG as second antibody. (B) Uninfected EPC cells as control (400 \times).

times, the membrane was visualized by enhanced chemiluminescence reagents.

Epitope mapping of MAbs

Four mutant proteins, KG-SVCV/Ma (1-186bp), KG-SVCV/Mb (169-354bp), KG-SVCV/Mc (337-522bp), and KG-SVCV/Md (505-672bp) (Fig. 1), were generated for basic epitope mapping.⁽¹¹⁾ The fragments were amplified from the EPC cells, which were infected by SVCV by a one-step RT-PCR. The primers for these fragments are listed in Table 1. Then the fragments were cloned into the vector pGEX-KG and transformed into competent *E. coli* BL21, respectively. After being induced by IPTG for 4 h, the bacterium was collected by centrifugation. The bacterial pellet was resuspended and sonicated to obtain the clear lysate. The four mutant proteins were purified and separated by 12% SDS-PAGE (Fig. 1) and transferred to the nitrocellulose membrane. Western blotting was used to analyze these four proteins according to the measures mentioned above.

Results and Discussion

Expression of M protein

The recombinant plasmid KG-SVCV/M was transformed into competent *E. coli* BL21 cells. The cells were induced by IPTG (1 mmol/L) for 3 h at 37 $^{\circ}$ C to expression M protein. The protein band (50 kDa) was detected by SDS-PAGE and Western blotting (Fig. 1).

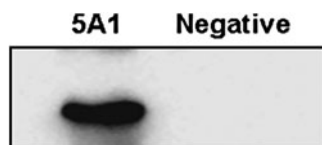


FIG. 3. Western blot analysis to evaluate MAb 5A1. (A) EPC cells were infected with SVCV for 36 h and specificity of monoclonal antibody 5A1 against M protein was analyzed by Western blot. Uninfected EPC cells were treated as negative control.

Generation of MAb against M protein of SVCV

During the immunization, blood from mice were collected and monitored by indirect ELISA. The mouse serum that showed the highest binding affinity was chosen for the last booster and cell fusion. One MAb 5A1 was isolated for further use.

Subtype identification of MAb against M protein of SVCV

The subtype of MAb was identified by the rapid ELISA mouse MAb isotyping kit. The result showed that MAb 5A1 belonged to the subtype IgG2b. The light chain of the MAb was kappa.

MAb specifically recognize M protein of SVCV

IFA and Western blotting were used to analyze the specificity of MAb 5A1. In IFA, MAb 5A1 showed positive reaction to SVCV-infected EPC cells but there were no fluorescence signals in the negative control cells (Fig. 2). The 24 kDa protein band was also detected by Western blotting (Fig. 3). In conclusion, MAb 5A1 was highly specific to SVCV.

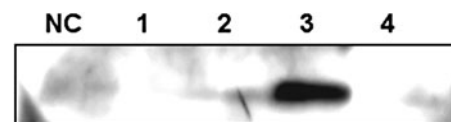


FIG. 4. Epitope mapping of MAb 5A1. Recombinant proteins KG-SVCV/Ma (1-186bp), KG-SVCV/Mb (169-354bp), KG-SVCV/Mc (337-522bp), and KG-SVCV/Md (505-672bp) were analyzed by Western blot to detect the specificity of 5A1. Bacilli precipitation of pGEX-KG was used as negative control. NC, negative control; lane 1, KG-SVCV/Ma (1-186bp); lane 2, KG-SVCV/Mb (169-354bp); lane 3, KG-SVCV/Mc (337-522bp); lane 4, KG-SVCV/Md (505-672bp).

Epitope mapping of MAb against SVCV

Recombinant M proteins KG-SVCV/Ma (1-186bp), KG-SVCV/Mb (169-354bp), KG-SVCV/Mc (337-522bp), and KG-SVCV/Md (505-672bp) were analyzed by Western blotting using 5A1. The results showed that the epitope that MAb 5A1 recognized is located in KG-SVCV/Mc (337-522bp) (Fig. 4).

Conclusion

In this study, BALB/c mice were immunized with prokaryotic expressed SVCV M protein. Using the hybridoma technique, one clone of MAb (5A1) was generated. The subtype of MAb was IgG2b and light chain was kappa. The specificity of MAb was analyzed by IFA and Western blotting. These results provide the material for future study of the functions of SVCV M protein.

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Author Disclosure Statement

The authors have no financial interests to disclose.

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