Monoclonal Antibodies Against G Protein of Spring Viremia of Carp Virus

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Part of the G protein (3094–4170 bp) of spring viremia of carp virus (SVCV) was expressed in *Escherichia coli* and purified by dialysis in our study. Two clones of monoclonal antibodies (MAbs 1H11 and 4B8) against G protein were generated by fusion of mouse myeloma cell line SP2/0 and spleen lymphocytes from part of G protein (3094–4170 bp) immunized mice. The results of ELISA (enzyme-linked immunosorbent assay), IFA (indirect immunofluorescent assay), and Western blot assay further demonstrated the characterizations of the two MAbs. Both 1H11 and 4B8 were specific to SVCV G protein. Ten pairs of synthesized overlapping peptides were used to identify the epitope of the MAbs. The MAbs are useful in the development of SVCV diagnostic methods.

Introduction

SPRING VIREMIA OF CARP VIRUS (SVCV) is the pathogen of infectious hemorrhagic swim bladder inflammation in common carp (*Cyprinus carpio*); the mortality of juvenile carp during spring outbreaks can be 30–70%.⁽¹⁾ Thus, SVCV has been listed as notifiable in the International Aquatic Animal Health Code of the Office International des Epizooties (OIE).^(2,3) The initial outbreak of spring viremia of carp was in European countries. Recently, it has been reported in the Middle East, the United States, China, Canada, and Brazil.⁽⁴⁾

The bullet-shaped SVCV is classified in the family Rhabdoviridae, genus *Vesiculovirus*. SVCV genome is situated in an elongated nucleocapsid, surrounded by a lipoprotein layer with glycoprotein projections.⁽⁵⁾ It contains a linear, negative-sense and single-stranded RNA, which encodes five structural proteins—nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and viral RNA-dependent RNA polymerase (L) in the order 3'-N-P-M-G-L-5'.^(6,7) The G protein trimetric peplomers or spikes on the virus surface that bind to cellular receptors and trigger viral endocytosis. It carries neutralizing epitopes and is a potential target for the development of DNA vaccines.⁽⁸⁾

In this study, we generated MAbs against G protein of SVCV. Because the whole genome of G protein is hard to express, nucleotide 3094–4170 (aa 1–359) of G protein was finally expressed and used as the immunized antigen.^(9,10) These specific MAbs can be useful tools for developing detection methods for SVCV and studying the function of G protein.⁽¹¹⁾

Materials and Methods

Cloning, expression, and purification of recombinant protein G

The G gene fragments (3094–4170 bp) were amplified from SVCV-infected *Epithelioma papulosum cyprini* (EPC) cells by a one-step RT-PCR. Primer sequences for the target genes are listed in Table 1. The target fragments were cloned into bacterial expression vector pGEX-KG. The recombinant plasmids SVCV-g-KG and the control plasmid pKG were transformed into competent *E. coli* BL21 cells and induced with isopropyl- β -thio-galactopyranoside (IPTG). After centrifugation, the bacterial pellet was resuspended and sonicated until a clear lysate was obtained. The target proteins were then purified by dialysis and stored at -80°C .⁽¹²⁾

Production of monoclonal antibody

Four-week-old female BALB/c mice were immunized subcutaneously with 100 μg of purified G protein at 2-week intervals. Three days before cell fusion, the mice were boosted with 200 μg of G protein. Three days later, mice splenocytes were harvested and fused with SP2/0 using 50% polyethylene glycol. The fused cells were cultured in HAT medium. Ten days later, the aminopterin was omitted and cells were cultured in HT medium. Hybridoma culture supernatants were screened by ELISA. The positive hybridoma lines were subcloned three times by limiting dilution method. The stable hybridoma clones were injected into liquid paraffin pretreated abdominal cavities of BALB/c mice. Subsequently, the MAbs were harvested and purified from the

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TABLE 1. PRIMER SEQUENCES

Gene	Primer orientation	Primer sequence (5'-3')
SVCV-g-KG	Forward	CGGGATCCATGTCTATCATCAGCTAC
	Reverse	GACTCGAGTTAGTCGACAATAGGTCCCTC
SVCV-gA-KG	Forward	AAAGGATCCATGTCTATCATCAGCTACAT
	Reverse	AAACTCGAGTTACCATCTATAATCACAA
SVCV-gB-KG	Forward	AAAGGATCCATGACTTGTGATTATAGA
	Reverse	AAACTCGAGTTATTTTCTCTGTCATTC
SVCV-gC-KG	Forward	AAAGGATCCATGGGCGAATGCAGAGAAA
	Reverse	AAACTCGAGTTAGGCTGTTTTCTCTACCC
SVCV-gD-KG	Forward	AAAGGATCCATGTGGGTAGAGAAAACAG
	Reverse	AAACTCGAGTTAGTCGACAATAGGTCCC

seroperitoneum with an antibody purification kit, according to the manufacturer's instructions.

Identification of MAb subtype

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

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

Immunofluorescence assay

In immunofluorescence assay (IFA), EPC cells were cultured in 24-well tissue culture plate (Costar Corning, Corning, NY) and inoculated with SVCV at 1 multiplicity of infection (MOI) when cells reached approximately 80% confluence. At 36 h post-infection, the cells were fixed with absolute methanol and processed for IFA using MAbs 1H11 and 4B8, followed by fluorescein isocyanate-conjugated goat anti-mouse IgG. Fluorescent images were examined under a fluorescent microscope.

Western blot analysis

To detect the specificity, the MAbs were analyzed by Western blot assay. SVCV-infected EPC cells were collected and separated by SDS-PAGE, then transferred to a nitrocellulose membrane. The membrane was blocked overnight with 1% bovine serum albumin (BSA) in TBST buffer (0.01 M Tris-HCl [pH 8.0], 150 mM NaCl, and 0.05% Tween-20), then incubated with 1:500 diluted MAbs 1H11 and 4B8 at 37°C for 1 h. After washing with TBST buffer three times, the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Southern Biotechnology, Birmingham, AL) secondary antibody (1:1000 diluted in blocking solution) at 37°C for 1 h. After washing three times, the protein bands were developed using an enhanced chemiluminescence system.

Epitope mapping of MAbs

Based on the epitope prediction by DNASTar, there are possibly 10 epitopes for G protein. To narrow the epitope scope, we expressed four mutant proteins that overlapped six amino acids with each other, without truncating the 10 possible epitopes. Their fragments were amplified from SVCV-infected

FIG. 1. SDS-PAGE analysis of recombinant proteins SVCV-g-KG, SVCV-gA-KG (1–270 bp), SVCV-gB-KG (253–522 bp), SVCV-gC-KG (505–774 bp), SVCV-gD-KG (757–1077 bp). (A) Lane 1, protein marker; lane 2, bacilli precipitation of pGEX-KG; lane 3, bacilli precipitation of SVCV-g-KG; lane 4, bacilli precipitation of SVCV-gA-KG (1–270 bp); lane 5, bacilli precipitation of SVCV-gB-KG (253–522 bp); lane 6, bacilli precipitation of SVCV-gC-KG (505–774 bp); lane 7, bacilli precipitation of SVCV-gD-KG (757–1077 bp). (B) Construction of recombinant plasmids expressing full length or truncated forms of G protein.

TABLE 2. DETECTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES

	1H11	4B8
ELISA titer	409,600	204,800
MAb subclass	IgG2a	IgG2b
Light chain	κ	κ

FIG. 2. Immunofluorescence staining (IFA) of SVCV-infected EPC cells with different MAb. After 36 h post-infection, cells were washed three times and fixed with 100% paraformaldehyde for 10 min, then blocked with BSA for 30 min. After three washes, cells were reacted with 1:100 diluted MAb for 1 h and incubated with Alexa Fluor 488 goat anti-mouse IgG for 30 min. Fluorescent images were examined with a fluorescent microscope. MAbs 1H11 and 4B8 against NS4B; uninfected EPC cells control (400 $\times$).

EPC cells by a one step RT-PCR. Subsequently, the target fragments were cloned into bacterial expression vector pEGX-KG. The recombinant plasmids SVCV-gA-KG (1–270 bp), SVCV-gB-KG (253–522 bp), SVCV-gC-KG (505–774 bp), and SVCV-gD-KG (757–1077 bp) were transformed into competent *E. coli* BL21 cells and induced with IPTG. After centrifugation, the bacterial pellet was resuspended and sonicated until a clear lysate was obtained. The target proteins were purified by dialysis and identified by SDS-PAGE (Fig. 1). The proteins were coated as antigen in ELISA assay for mapping epitope.

Based on the ELISA result, ten partially overlapping in length of 15 amino acids' short peptides covering 757–1077 bp of G protein were synthesized. Further epitope mapping assay was investigated using these peptides as ELISA coating antigen.

Results and Discussion

G protein expression in Escherichia coli

The recombinant plasmid SVCV-g-KG was transformed into competent *E. coli* BL21 cells and induced with 1 mmol/L IPTG at 37°C for 3 h to express the recombinant protein. A protein band of 66 kDa was detected by SDS-PAGE and Western blot, which corresponded with the molecular weight of the fusion protein (Fig. 1).

Generation of MAbs against G protein of SVCV

During immunization, mice blood samples were collected and monitored by indirect ELISA; one showed high binding affinity was chosen for the last booster and further cell fusion. After three subclones by limiting dilution method, two

MAbs 1H11 and 4B8 were finally isolated and expanded for further use.

Subtype identification of MAbs against G protein of SVCV

The subtype of the MAbs was identified by a rapid ELISA mouse MAb isotyping kit. The result showed that both 1H11 and 4B8 belong to the subtype IgG2b. The light chains of these MAbs were kappa (Table 2).

MAbs specifically recognize G protein of SVCV

The specificity of MAbs was identified by IFA and Western blot assay. In IFA, both MAbs 1H11 and 4B8 showed positive reaction to SVCV-infected EPC cells but no fluorescence signals were observed in the negative control cells (Fig. 2). Because of carbohydrate, a 80 kDa protein band was seen in the Western blot, which differs from the calculated size of 57.4 kDa⁽¹⁰⁾ (Fig. 3). In conclusion, MAbs 1H11 and 4B8 were highly specific to SVCV.

Epitope mapping of MAbs against SVCV

Recombinant proteins SVCV-gA-KG (1–270 bp), SVCV-gB-KG (253–522 bp), SVCV-gC-KG (505–774 bp), and SVCV-gD-KG (757–1077 bp) were coated as antigen separately. The ELISA result showed MAbs 1H11 and 4B8 reacted with SVCV-gD-KG (757–1077 bp) (Table 3). Then, a set of synthesized peptides was coated as antigen in ELISA for further epitope mapping. Finally, the amino acids 273–287 (DGTLSVSGHRPGLDLI) were identified as the epitope of G protein (Table 4). This result may be useful for the development of diagnosis and detection methods of SVCV.

FIG. 3. Specificity of monoclonal antibodies against G protein analyzed by Western blot assay.

TABLE 3. EPITOPE SCREENING OF MAbs AGAINST G PROTEIN OF SVCV BY ELISA

Proteins	Value of OD ₆₃₀ 1H11	Value of OD ₆₃₀ 4B8
SVCV-gA-KG (1–270 bp)	0.0631	0.0946
SVCV-gB-KG (253–522 bp)	0.0538	0.0574
SVCV-gC-KG (505–774 bp)	0.1507	0.0886
SVCV-gD-KG (757–1077 bp)	2.1574	2.2298
Negative control	0.0683	0.0817

TABLE 4. EPITOPE SCREENING OF MABS AGAINST G PROTEIN OF SVCV BY ELISA

<i>Synthesized peptide</i>	<i>Value of OD630 1H11</i>	<i>Value of OD630 4B8</i>
WVEKTAGTLTTIHDN	0.0562	0.0551
TIHDNVPKCV DGLV	0.0533	0.0561
DGLTVSGHRPGLDLI	2.7114	2.4048
GLDLITVFNLNVV	0.0528	0.0533
LENVVEYTLCEGTR	0.0522	0.1092
EGTKRKINKQEKLTS	0.0538	0.0586
EKLTSVDLSYLAPRI	0.1044	0.0532
LAPRIGGFGSVFRVR	0.0647	0.0527
VFRVRNGTLERGSTT	0.0645	0.062
RGSTTYIRIEVEGPIVD	0.0584	0.0601
Negative control	0.0328	0.0698

Conclusion

In this study, BALB/c mice were immunized with prokaryotic expressed G protein of SVCV. Through hybridoma technique, two clones of MAb (1H11 and 4B8) were generated. The subtype of MAb was IgG2b and light chain was kappa. IFA and Western blot assay proved the specificity of MAb. These results provide the information for future development of detection methods and research of the pathogenic mechanism of SVCV.

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Author disclosure statement

The authors have no financial interests to disclose.

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