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Original article

Identification of a linear epitope in the capsid protein of goose astrovirus with monoclonal antibody

G. Dai^{1,2,4}, X. Huang^{1,4}, Q. Liu^{1,4}, Y. Li^{1,4}, L. Zhang^{1,4}, K. Han^{1,4}, J. Yang^{1,4},
Y. Liu^{1,4}, F. Xue², D. Zhao^{1,2,3,4}

¹ Institute of Veterinary Medicine,
Jiangsu Academy of Agricultural Sciences, No. 50 Zhongling Street,
Nanjing City, Jiangsu Province, 210014, PR China

² College of Veterinary Medicine,
Nanjing Agricultural University, No. 1 Tongwei Road, Nanjing City, Jiangsu Province 210095, PR China

³ Institute of Life Sciences,
Jiangsu University, No. 301 Xuefu Road, Zhenjiang, Jiangsu Province, 212013, PR China

⁴ Key Laboratory of Veterinary Biological Engineering and Technology,
Ministry of Agriculture, Nanjing City, Jiangsu Province, 210014, PR China

Abstract

Goose astrovirus (GoAstV) is a novel avastrovirus that typically causes gosling gout and results in 2 to 20% mortality. GoAstV capsid protein is the sole structural protein, which is responsible for viral attachment, assembly, maturation as well as eliciting host antibodies. However, the epitopes within capsid protein have not been well studied. In this study, a monoclonal antibody, named 1D7, was generated against GoAstV capsid protein by hybridoma technology. Western blot results showed that this MAb could react with recombinant capsid protein expressed in *E. coli*. Also, it recognized the precursor of capsid protein, VP90 and VP70, in GoAstV-infected cells. Besides, excellent specificity of MAb 1D7 was further demonstrated in indirect immunofluorescence assay and immunohistochemical analysis. Epitope mapping results revealed that MAb 1D7 recognized the epitope ³³QKVY³⁶ within Cap protein. Sequence alignment indicated that ³³QKVY³⁶ is a conserved epitope among the isolates of goose astrovirus type 2 (GoAstV-2), suggesting the potential for its use in GoAstV-2 specific diagnostic assay. These findings may provide some insight into a function of the GoAstV capsid protein and further contribute to the development of diagnostic methods for GoAstV infection.

Key words: goose astrovirus, capsid protein, monoclonal antibody, epitope

Introduction

Goose astrovirus (GoAstV) is a novel astrovirus that been isolated and reported in China in 2018 (Zhang et al. 2018). Infection of GoAstV causes depression, loss of appetite, lethargy, and white feces. Autopsies showed that severe urate deposition in the internal organs, especially the heart, liver and kidney. Pathological damage was most severe to the kidney, which was swollen and hemorrhagic (Yin et al. 2021, Zhu and Sun 2022). The mortality of GoAstV was 2 to 20% in 4- to 16-day old gosling (Huang et al. 2021). The GoAstV infection spread out in most goose-farming regions in China including Jiangsu, Shandong, Anhui, Guangdong, and Zhejiang provinces. The epidemic of GoAstV leads to huge economic losses in domestic goose breeding industry.

GoAstV belongs to Astroviridae family, and its genome contains three open reading frames (ORF1a, ORF1b and ORF2; Yang et al. 2018). The structural protein (capsid protein) is expressed from subgenomic RNA produced after replication that contains ORF2, which is the most variable region of genome (Fuentes et al. 2012). The GoAstV capsid protein (Cap) is the sole structural protein and mainly responsible for the assembly of virus particles. Cap is synthesized as an inactive precursor protein (VP90) that undergoes successive cleavage steps. The carboxy terminus of VP90 is cleaved by intracellular caspases to generate VP70, of which 180 copies form an immature virion. It is reported that caspase cleavage is essential for virion release. The VP70 is further hydrolyzed to VP34, VP27 and VP25 by extracellular trypsin-like proteases to form mature, infectious virions (Banos-Lara and Méndez 2010). Folded VP34 constitutes the shell of the virus (capsid core), while VP27/VP25 form capsid spikes (spike domain). Heterodimeric VP27/VP25 associates with capsid through the interaction between VP27 and capsid, while VP25 is easily released from virus particle because it is not expected to be anchored to the capsid core (Aguilar-Hernández et al. 2018).

In addition to playing crucial roles in viral assembly, virion maturation and infectivity, Cap also relates to virus attachment phase in viral infection. The neutralizing monoclonal antibodies that react with the Cap spike domain were able to block attachment of astrovirus to target cells, which suggests that Cap spike domain contains a receptor-binding site (Bass and Upadhyayula 1997).

Besides, Cap contains antigenic determinant of this virus and both regions of the Cap can elicit host antibodies target astrovirus. It is demonstrated that the polyclonal antibodies produced by immunization with mature astrovirus reacts with all three fragments of the

astrovirus capsid protein, VP34, VP27 and VP25 (Bass and Qiu 2000). The spike domain had 5 to 10-fold higher antibody reactivity compared to the capsid core, suggesting that the spike domain is the principal antigen on the astrovirus surface (Arias and DuBois 2017).

However, there is little knowledge about the epitopes of GoAstV Cap. In trying to screen and identify epitopes within Cap of GoAstV, we have generated monoclonal antibody (MAb) from mice immunized with purified recombinant GoAstV Cap, and identified a linear B-cell epitope recognized by MAb through the screening of overlapping truncated Cap fragments. This study may advance our understanding of GoAstV Cap structure and its interaction with antibody.

Materials and Methods

Cells and viruses

Leghorn Male Hepatoma cell line (LMH) were grown in DMEM containing 10% FCS at 37°C under an atmosphere of 5% CO₂. The myeloma cell line SP2/0 was cultured in RPMI 1640 medium containing 10% FCS in the same condition. The GoAstV-AHQJ18 isolate was isolated from an infected goose with gout and kept in our laboratory.

Expression of recombinant Cap protein

The full-length gene fragment of GoAstV Cap protein was amplified by PCR using the primers Cap-F (5'-CAGCAAATGGGTCGCGGATCCATGGCAGA CAGGGCGGTGGC-3') and Cap-R (5'-TGGTGGT GCTCGAGTGC GGCCGCCTCATGTCCGCCCTT CTCAA-3'). The BamHI and Not I restriction sites are italicized. The PCR product was cloned into the BamHI and Not I digested prokaryotic expression vector pET28a, and then transformed into *E. coli* BL21 for expression. After induction by IPTG at 37°C for 6 h, the expression and distribution of the recombinant Cap protein was analyzed by SDS-PAGE. The proteins were purified by BeyoMag Anti-His Magnetic Beads (Beyotime) according to the instructions.

Production of MAb

For production of MAb against Cap protein, 6- to 8-week-old BALB/c mice were immunized subcutaneously with 100 µg of purified recombinant Cap protein. Initial immunization was with Freund's complete adjuvant, followed by two boosts with incomplete adjuvant at two weeks interval. At two weeks after the last immunization, the sera were collected to determine the titers of antibody by indirect ELISA. Three days prior to fusion, mice received 100 µg of purified recombinant

Table 1. Primers used in epitope mapping.

Fragment name (Position)	Sequence (5'-3')	Fragment name (Position)	Sequence (5'-3')
A (1-280 aa)	GCTGATATCGGATCCGGATCCATGGCAGACAGGGCCGTGGC TGGTGGTCTCGAGTGCAGTCCGGCCGACCACTCTGGGTATTAC	L8 (29-204 aa)	GCTGATATCGGATCCGGATCCAAAGAACCAAGCAG TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC
B (248-494 aa)	GCTGATATCGGATCCGGATCCATTCGCAACTATGGTCC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L9 (31-204 aa)	GCTGATATCGGATCCGGATCCCAAAAGCAGAAAGT TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC
C (461-704 aa)	GCTGATATCGGATCCGGATCCATACAAATGCAAAATAACCTT TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L10 (33-204 aa)	GCTGATATCGGATCCGGATCCCAAAAGTATATAAGC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC
S1 (1-117 aa)	GCTGATATCGGATCCGGATCCATGGCAGACAGGGCCGTGGC TGGTGGTCTCGAGTGCAGTCCGGCCGCTTGTGAAGCCCTTATACCTTAG	L11 (34-204 aa)	GCTGATATCGGATCCGGATCCAAAGTATATAAGCC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC
S2 (87-204 aa)	GCTGATATCGGATCCGGATCCGCAAGCAATAATAC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L12 (35-204 aa)	GCTGATATCGGATCCGGATCCGTATATAAGCCCA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC
S3 (174-280 aa)	GCTGATATCGGATCCGGATCCGGCTCAAACACTTTTGG TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L13 (1-34 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTCTGCTTTGGT
S4 (19-204 aa)	GCTGATATCGGATCCGGATCCGTGGTCACTGTTAAGA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L14 (1-35 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTCTGCTTTGG
S5 (45-204 aa)	GCTGATATCGGATCCGGATCCGGCAGAGGAAGCTTGG TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L15 (1-36 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTCTGCTTTGG
S6 (67-204 aa)	GCTGATATCGGATCCGGATCCGCAAAATGACTACAC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L16 (1-37 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L1 (20-204 aa)	GCTGATATCGGATCCGGATCCGTCACCCGTTAAGAAAA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L17 (1-38 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L2 (21-204 aa)	GCTGATATCGGATCCGGATCCACCCGTTAAGAAAA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L18 (1-39 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L3 (22-204 aa)	GCTGATATCGGATCCGGATCCGTTAAGAAAA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L19 (1-40 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L4 (23-204 aa)	GCTGATATCGGATCCGGATCCCAAGAAAAACACCCAA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L20 (1-41 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L5 (24-204 aa)	GCTGATATCGGATCCGGATCCAAAAACACCCAAAA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L21 (1-42 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L6 (25-204 aa)	GCTGATATCGGATCCGGATCCAAACACCCAAAAAGGA TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC	L22 (1-43 aa)	GATCTGGTCCGGTGGATCCATGGCAGACAGGGCCGTGGC TCAGTCAGTCACCATCCGGCCGCTTATATACCTTTCTGC
L7 (27-204 aa)	GCTGATATCGGATCCGGATCCCAAAAAAGAAAC TGGTGGTCTCGAGTGCAGTCCGGCCGCTCAGTGGGTGAATC		

Cap protein without adjuvant. Fusion of mouse splenocytes with myeloma cell line SP2/0 by using the standard method. The fused cells were cultured in the selective medium for two weeks, and then cell supernatants were screened by indirect ELISA. The MAb-producing hybridomas were subcloned three times by limiting dilution. The hybridoma of interest was intraperitoneally inoculated into BALB/c mice and ascitic fluid was harvested for purification by Protein L Magnetic Beads (Invitrogen).

Characterization of MAb

The isotype of MAb was determined by Mouse Monoclonal Antibody Isotyping Reagents (Sino Biological) according to the manufacturer's instructions. To investigate the antigen binding of the generated MAb, the lysate of GoAstV-infected LMH cells and purified recombinant Cap protein were subjected to SDS-PAGE followed by Western blotting. Indirect immunofluorescence assay (IFA) was performed as follows. LMH cells were infected with GoAstV and fix with chilly methanol-acetone (1:1, v/v) at 48 h post infection. The fixed cells were incubated with MAb at 37°C for 1 h, followed by 1 h incubation with Alexa Fluor 488-labeled goat anti-mouse IgG. The cells were then detected using fluorescent microscope.

Immunohistochemical analysis

1-day-old goslings were injected intramuscularly with 10^5 TCID₅₀ of GoAstV. The control group was given equal volume of PBS. At 7 days post injection, goslings were euthanized with intravenous pentobarbital sodium. The spleen, liver and kidney were collected and fixed in 4% paraformaldehyde for histopathological and immunohistochemical examination.

MAb sequencing

MAb hybridoma cells were harvested for total RNA extraction using the Trizol plus RNA purification kit (Invitrogen). The genes encoding heavy- and light-chain variable region of MAb were amplified by HiScript II one step RT-PCR kit (Vazyme) using specific primers. The primers used were as follows: VHF: 5'-AGRTBSARCTKCAGSAGTCWGG-3', VHR: 5'-TGMRGAGACDGTGABHRDRGTBCCTG RCCCCAG-3'; VLF: 5'-GGGAATTCATGGAYRTYN WGATGACNCARWCT-3', VLR: 5'-GGGGATCCTA CAGTTGGTGCAGCATCAGCCCG-3'. The purified PCR products were cloned into pMD-18T for DNA sequencing and BLAST analysis.

Epitope mapping

In order to roughly locate the epitope recognized by MAb, a series of truncated overlapping fragments derived from GoAstV Cap were expressed using the *E. coli* expression system as described above. The primers used to amplify the genes of truncated overlapping fragments are listed in Table 1. PCR products were inserted into the pET32a vector using the BamHI and Not I restriction sites (italicized in Table 1). To map the minimal epitope, additional truncated fragments derived from 1-44 aa of the GoAstV Cap were cloned into pGEX-4t-1 with GST tags. The positive recombinant plasmids were transformed into *E. coli* BL21 competent cells. The fusion proteins were expressed by induction of 1 mM IPTG for 6 h at 37°C and then used to determine the epitopes recognized by MAb using indirect ELISA and Dot blot.

Indirect ELISA

A 96-well ELISA plate was coated with the recombinant protein at a concentration of 4 µg per well

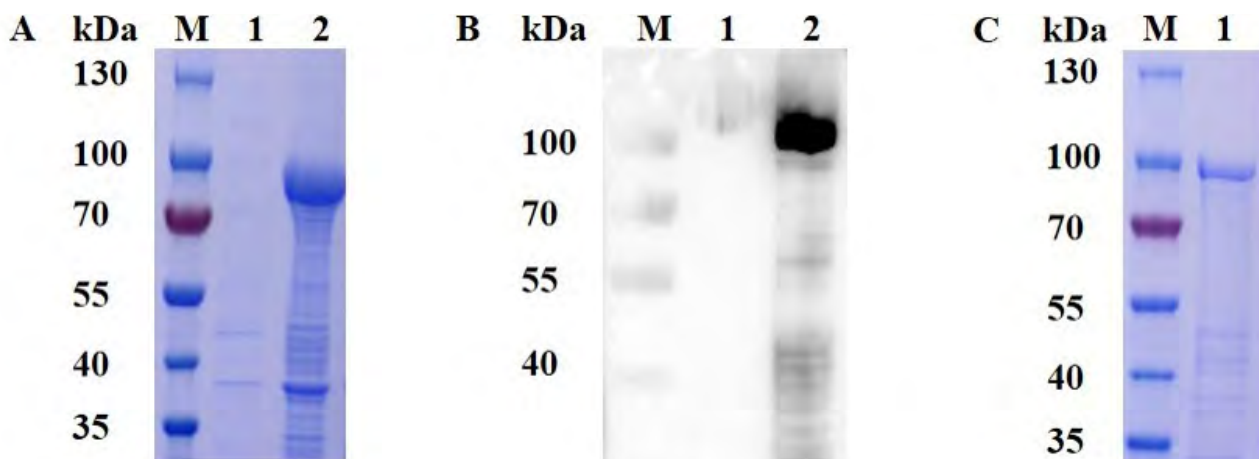


Fig. 1. Expression and detection of recombinant goose astrovirus (GoAstV) Cap protein. A, induction of Cap protein from *E. coli* BL21. Lane 1, soluble supernatant after sonication. Lane 2, inclusion bodies collected after sonication. B, detection of recombinant Cap protein with anti-His-tag antibody. Lane 1, crude lysates from *E. coli* BL21 with pET-28a vector. Lane 2, recombinant Cap protein is recognized by anti-His-tag antibody. C, detection of Cap protein after purification. Lane 1, purified recombinant Cap protein.

at 4°C overnight. After washing three times with PBST (PBS containing 0.05% Tween-20), 100 µL of MAb was added into each well and incubated at 37°C for 1 h. Then MAb was removed, and the plate was washed three times with PBST. 100 µL of HRP-labeled goat anti-mouse IgG was added into each well and incubated at 37°C for 1 h. After further washing, 100 µL of HRP substrate was added into each well and incubated for 10 min at room temperature. The reaction was stopped by the addition of 50 µL per well of 2 M H₂SO₄. Absorbance at 450 nm was measured with a BioTek microplate reader.

Dot blot

Approximately 1 µg of each truncated protein was spotted onto the nitrocellulose membrane. After blocking, the membrane was incubated with MAb at 37°C for 1 h. Between incubation steps, the membrane was washed three times with PBST. Mouse alkaline phosphatase (AP) secondary antibody was added onto the membrane and incubated at 37°C for 1 h, followed by washing as above. AP substrate was then added onto the membrane and incubated in the dark for 10 min at room temperature until the appearance of spots.

Sequence analysis

The sequences of astrovirus strains were collected from GenBank. To evaluate the level of conservation of the MAb epitope among different astroviruses, amino acid sequence alignments of identified epitope were performed using Lasergene MegAlign software.

Statistical analysis

The experiments were performed in triplicate. The data are expressed as mean ± SD (standard deviation)

of three independent experiments. Statistical analysis was performed using the SPSS software. Values of *p* below 0.05 were considered to be statistically significant.

Results

Expression, identification and purification of GoAstV Cap protein

As shown in Fig. 1, the recombinant GoAstV Cap protein was expressed as inclusion bodies. The apparent molecular mass of His-tagged Cap fusion protein was approximately 90 kD, closely matched with the calculated value based on primary structure. Western blotting showed that the fusion protein could be combined with anti-His-tag antibody, confirming the expression of GoAstV Cap protein in *E. coli*. The recombinant Cap protein was homogeneous after purification using anti-His magnetic beads.

Production and characterization of MAb against GoAstV Cap protein

After cell fusion and screening, one MAb of highest affinity was generated and named as 1D7. The heavy chain of 1D7 was IgM and the light chain was κ type. The Western blotting showed that 1D7 could recognize the recombinant GoAstV Cap protein (Fig. 2A). The lysate of GoAstV-infected LMH cells was also used in Western blotting. MAb 1D7 could react with VP90 and VP70 (Fig. 2B). The IFA results demonstrated that 1D7 has a positive reaction to GoAstV. No fluorescence staining was observed in negative control cells (Fig. 2C). It is suggested that it can be used for detecting Cap protein in GoAstV-infected cells by IFA.

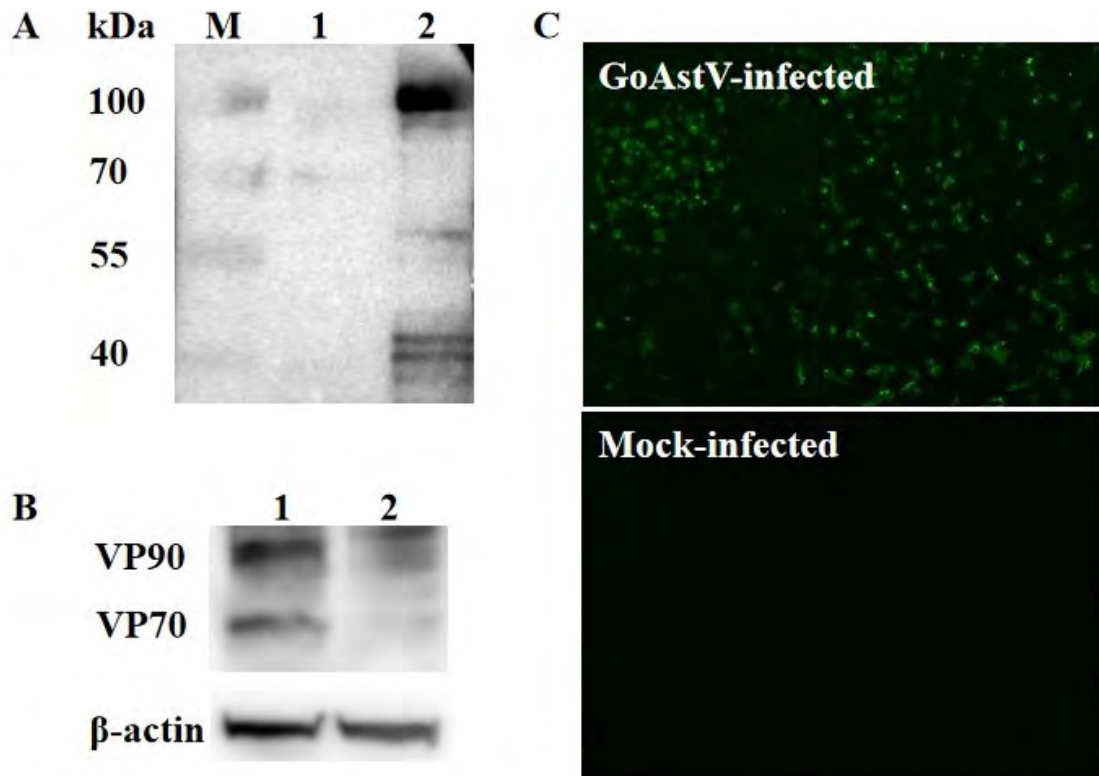


Fig. 2. Characterization of MAb 1D7 against GoAstV Cap protein. A, MAb 1D7 reacted with recombinant GoAstV Cap protein. Lane 1, crude lysates from *E. coli* BL21 with pET-28a vector. Lane 2, purified recombinant Cap protein. B, MAb 1D7 recognized VP90 and VP70 in the lysate of GoAstV-infected LMH cells. Lane 1, GoAstV-infected cells. Lane 2, mock-infected cells. C, immunofluorescence analysis of GoAstV Cap protein in GoAstV-infected LMH cells using MAb 1D7.

Immunohistochemical analysis

The results showed that positive signals were observed in kidney, liver and spleen from GoAstV-infected goslings. GoAstV antigen could be detected in the renal tubules, collecting ducts and renal corpuscle of the kidney, in the nuclei of hepatocytes, biliary epithelial cells and central vein endothelial cells of the liver, and in the nuclei of splenic lymphocytes. In contrast, no viral antigen was observed in kidney, liver and spleen from goslings of the control group. These are consistent with the hispathological damagen pattern observed in HE staining (Fig. 3).

Sequence analysis of heavy- and light-chain variable region genes of MAb

The length of the cloned MAb heavy chain variable region was 321 bp and that of the light chain variable region was 348 bp. The complementary determining regions (CDR) and framework regions (FR) were defined according to the Kabat numbering scheme. The CDRH3 of MAb 1D7 has 12 amino acids (SSYYGSSCAMDY).

Identification of epitope recognized by MAb

The expressed truncated proteins were analyzed with MAb by indirect ELISA and Dot blot (Fig. 4).

The reactivities of three recombinant proteins (fragments A, B and C) to MAb showed that only fragment A (1-280 aa) reacted with 1D7, demonstrating that the epitope is located in the N-terminal part of GoAstV Cap. Subsequently, three overlapping recombinant proteins (fragments S1, S2 and S3) derived from fragment A were used to identify the epitope. The results showed that only fragment S1 (1-117 aa) exhibited positive reactivity to MAb. Next, three truncated recombinant fragments (fragments S4, S5 and S6) were used as antigen to test the reactivities of MAb. The results showed that MAb could react with S4, but not react with S5 and S6. Based on the above findings, MAb recognized the epitope of 19-44 aa. Then we expressed and purified a set of truncated recombinant protein (fragments L1 to L22). In the indirect ELISA assay, it is found that the MAb recognized all the fragments except L11, L12, L13 and L14. These results were further confirmed by Dot blot. Therefore, ³³QKVY³⁶ is the minimal amino acid sequence required for recognition by MAb 1D7.

Sequence analysis of the identified epitope in Astrovirus strains

The alignment of the amino acid sequence of the Avastrovirus strains demonstrated that the epitope

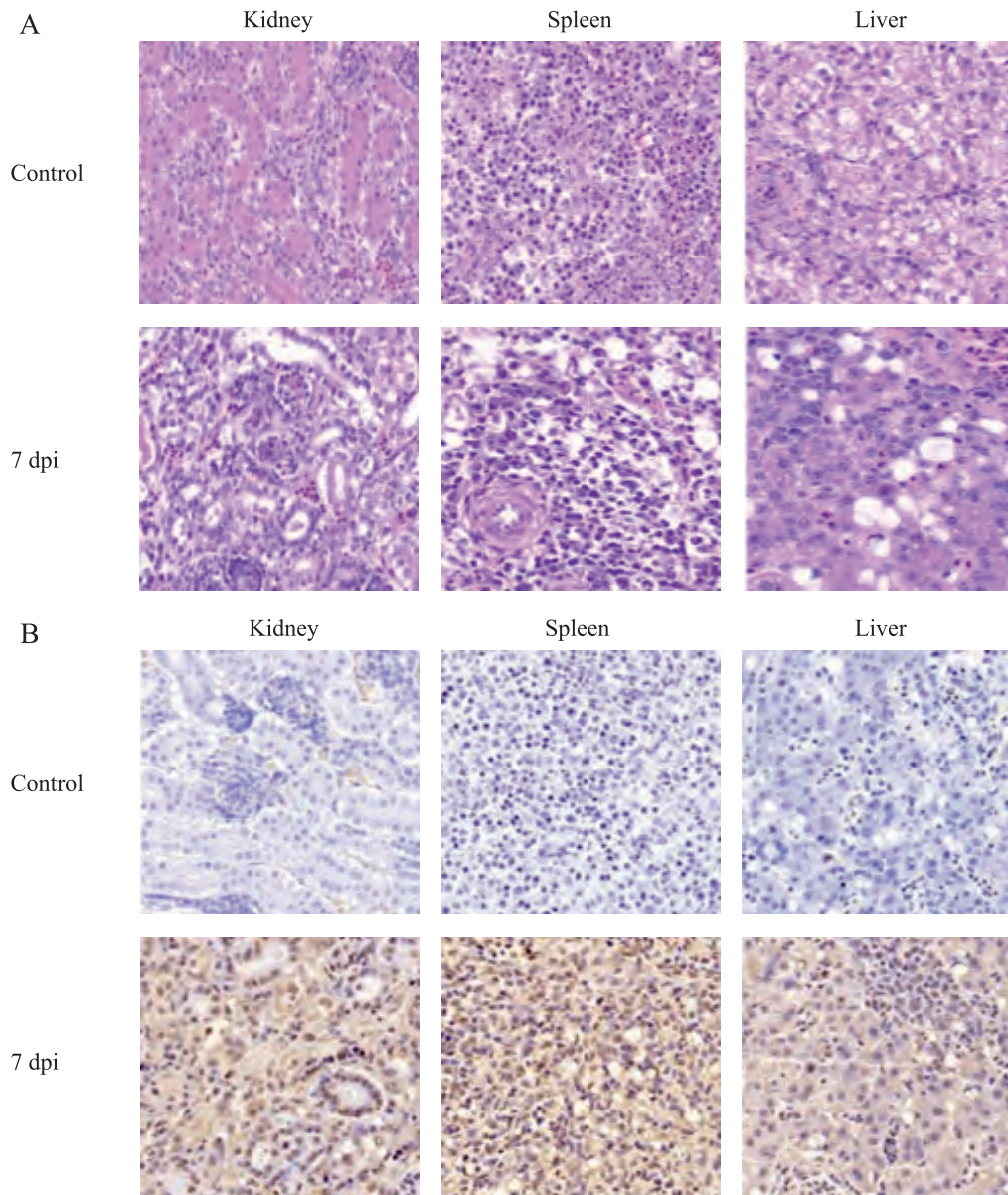


Fig. 3. The MAb 1D7 was analyzed in kidney, in spleen and in liver by immunohistochemical analysis. Stained with hematoxylin and eosin (A) and immunohistochemically (B), respectively.

$^{33}\text{QKVY}^{36}$ was completely conserved in all the GoAstV-2 strains. However, it was highly divergent from GoAstV-1, CAstV and DAstV strains (Fig. 5). The high homology of the $^{33}\text{QKVY}^{36}$ suggests that it is a conservative epitope of GoAstV-2 strains.

Discussion

GoAstV is a newly emerging virus that severely threatens goslings and cause huge losses to the breeding industry of goose. To date, there no vaccine and specific treatments available for GoAstV infection. The Cap protein, an important structural protein, is the major antigen responsible for eliciting antibody. However, the antigenic structure of Cap has not been well defined.

Developing monoclonal antibodies against the Cap protein is essential for elucidating biological properties of Cap protein.

In this study, a monoclonal antibody recognized GoAstV Cap protein was generated. MAb 1D7 could react with recombinant Cap protein, VP90 and VP70. The immunohistochemical analysis showed that MAb has a specificity for recognizing GoAstV in sections from liver, spleen and kidney tissues. The results are consistent with the results reported previously. Huang et al. found that the viral load reaching a peak at 7 dpi in the kidney and a large number of positive signals were detected in renal tubular epithelial cells (Huang et al. 2021). Besides, Wu et al. reported that few positive signals were also located in the splenic lymphocytes and macrophages (Wu et al. 2021). In this study,

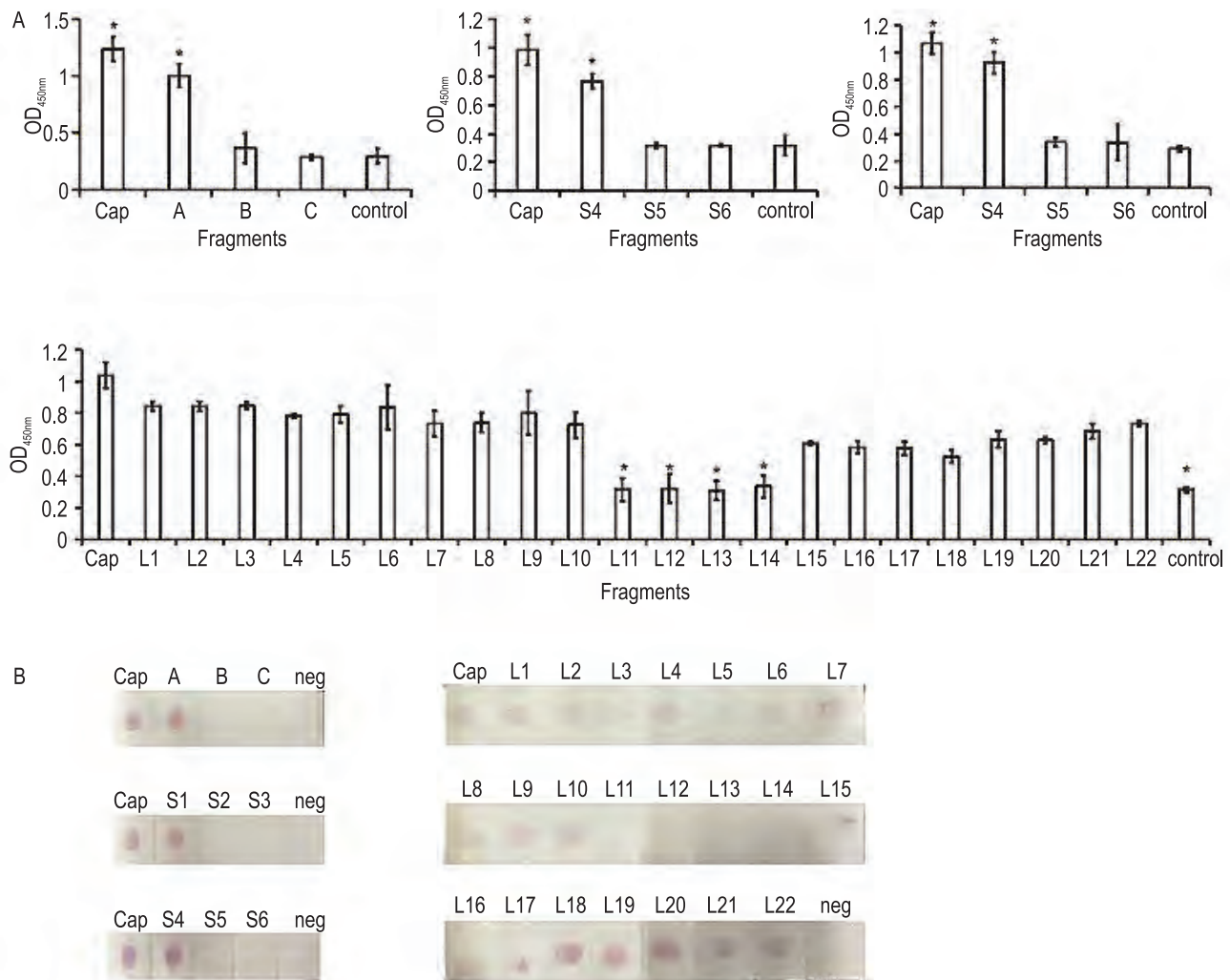
Identification of a linear epitope in the capsid protein of goose ...


Fig. 4. Epitope mapping of MAb 1D7 by indirect ELISA (A) and Dot blot (B). The asterisk (*) represents a statistically significant difference ($p < 0.05$).

our MAb could not only recognize GoAstV antigen in the spleen and kidney, but also recognize GoAstV antigen in the hepatocytes, biliary epithelial cells and central vein endothelial cells in liver. These results suggested that GoAstV has a wide tissue tropism and its target organs included liver, spleen and kidney, which was in accordance with those in other studies (Yin et al. 2021).

Monoclonal antibody has the ability to recognize unique binding site on the specific antigens. Therefore, monoclonal antibody is often used to identify epitopes of viral proteins. For astrovirus, several MAbs against Cap protein were produced and used for screening valuable epitopes within Cap protein (Lan et al. 2019, Ricemeyer et al. 2022). However, the epitopes of GoAstV have not been well identified. In the present study, MAb 1D7 against GoAstV Cap protein was used for epitope mapping. The ELISA and Dot blot revealed that 1D7 could react with the epitope ³³QKVY³⁶ within Cap protein.

Although the MAb is shown to be highly specific

for GoAstV by IFA and immunohistochemical analysis, it did not show in vitro neutralizing activity against GoAstV (data not shown). The reason may be that the epitope ³³QKVY³⁶ recognized by MAb is located in a region of basic amino acids at the N-terminus. This region, spanning residues 1 to 70, is thought to be an RNA coordination motif which is exposed within the virion and involved in packaging of the viral RNA (Toh et al. 2016). Although deletion of the region from 31 aa to 50 aa did not influence capsid protein package, the infectivity of viral particles was severely decreased. Therefore, the knowledge of the fate of N-terminal region during and after capsid protein processing is important for elucidating astroviral assembly (Geigenmüller et al. 2002). However, it is hampered by the lack of antibody that recognize the epitopes close to the N-terminus (Caballero et al. 2004). The MAb 1D7 generated herein can be used to study the N-terminal portion, for this reason, MAb 1D7 may be helpful for unraveling the structure and biological functions of GoAstV capsid protein.

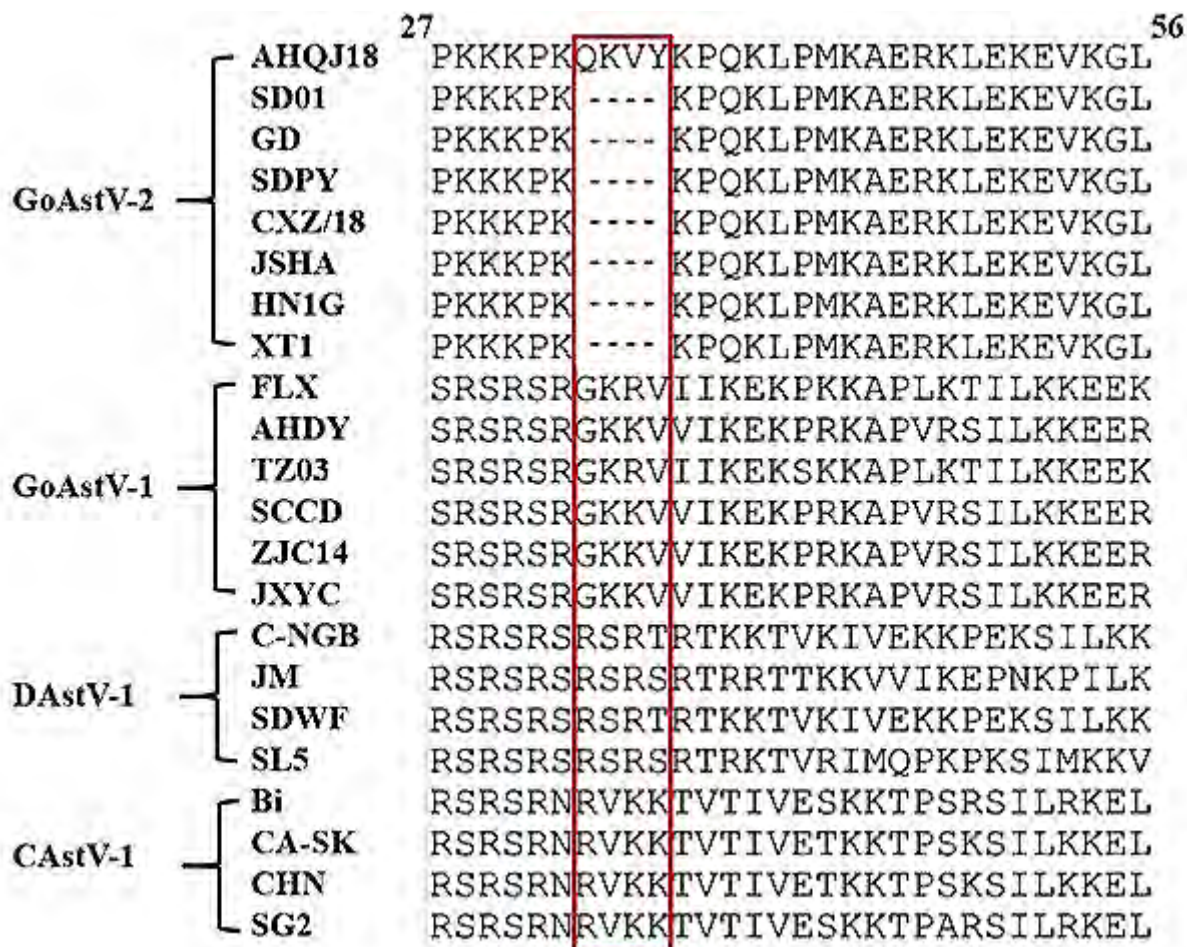


Fig. 5. Sequence alignment of the epitope with the corresponding regions from other avastrovirus: GoAstV-1, CAstV and DAstV. The red box indicates the epitopes of different Cap proteins.

Basing on the phylogenetic relationship of the Cap gene, GoAstV can be divided into two distinct phylogenetic clades: goose astrovirus type 1 (GoAstV-1) and goose astrovirus type 2 (GoAstV-2). All the GoAstV-2 strains are associated with the outbreak of goose gout, while GoAstV-1 strains are reported in geese with enteritis and gout (Wang et al. 2021). In fact, concurrent infection of GoAstV-1 and GoAstV-2 is not uncommon. A previous study showed that 45.66% of GoAstV-1 positive samples were proved to be positive for GoAstV-2, and GoAstV-1/GoAstV-2 co-infection may result in significant mortality in gout goslings (Zhang et al. 2022). Here, the analysis of the amino acid sequences of different GoAstV strains revealed that ³³QKVY³⁶ in Cap protein was conserved among GoAstV-2 strains, indicating that this MAb may be used in specific diagnostic assay for GoAstV-2.

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