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Identification of a novel linear B-cell epitope recognized by a monoclonal antibody against the capsid protein of goose astrovirus genotypes 2

Haiqin Li[1], Haoyu Huang[1], Cui Lin[1], Fanfan Zhang[1], Jia Tan[1], Na Li[1], Chengcheng Wu[1], Jiangnan Huang[1], Qipeng Wei[1], Siyi Wang[1], Yujun Zhu[2*] and Yanbing Zeng[1*]

Abstract

Background Goose astrovirus genotype 2 (GoAstV-2) is a novel and highly infectious pathogen characterized by symptoms of gout, swelling, and hemorrhage of the kidneys in 5–15-day-old goslings. The virus has caused severe economic losses for the goose husbandry industry. The capsid protein of GoAstV-2 is involved in viral packaging and immunogenicity, and it has served as a target antigen for diagnostic methods.

Results In this study, the N-terminus of the GoAstV-2 Cap protein (rCap) was successfully expressed and used to immunize BALB/c mice. A new monoclonal antibody (mAb) named 7B2 against the GoAstV-2 rCap protein was prepared. Immunofluorescence assay and Western blot analysis showed that mAb 7B2 had a strong binding affinity to the GoAstV-2 rCap protein. The results of epitope mapping demonstrated that the minimum linear epitope recognized by mAb 7B2 was located in the amino acids ^{153}NTAGPESIDT^{162}. Sequence homology analysis revealed that this epitope was highly conserved among the GoAstV-2 strains analyzed and that it showed 90% sequence similarity with turkey astrovirus 2 strains and low similarity (less than 50%) with other astroviruses.

Conclusions These findings will be useful for the development of diagnostic kits for GoAstV-2 and will aid further research on the structure and function of the capsid protein.

Keywords Goose astrovirus genotypes 2, Cap protein, Monoclonal antibody, B-cell linear epitope

Background

A novel goose astrovirus (GoAstV) is the causative agent of gout disease in geese [1]. The disease was first reported in Anhui Province, China, in 2015 and rapidly spread to other provinces of China within a short time [2–5]. GoAstV is classified into two genotypes, GoAstV-1 and GoAstV-2, with low genomic homology and antigenicity [6, 7]. The typical symptoms of urate deposition in the internal organs, swelling, and hemorrhage in the kidneys are observed in 5–15-day-old geese infected with GoAstV-2, and the mortality rate of this disease is as high as 50% [8–10]. This has led to significant economic losses for the goose industry in China Due to the lack of specific treatments and effective vaccines, GoAstV-2 remains prevalent in commercial flocks.

GoAstV, a relatively new member of the *Astroviridae* family, is a small, nonenveloped, positive-sense,

*Correspondence:
Yujun Zhu
837895642@qq.com
Yanbing Zeng
zengyb2004@163.com
[1] Institute of Animal Husbandry and Veterinary Medicine, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi 330200, China
[2] Guangdong Provincial Biotechnology Research Institute, Guangzhou, Guangdong 510633, China

single-stranded RNA virus [7, 11, 12]. The complete genome sequence of GoAstV was first published in 2017 and is approximately 7.2 kb in length [13, 14]. The genomic structure of GoAstV is similar to those of other astroviruses, containing a 5'-untranslated region (UTR), a 3'-UTR, a poly (A) tail, and three open reading frames (ORF1a, ORF1b, and ORF2) [15]. ORF1a and ORF1b are responsible for encoding non-structural proteins associated with genomic replication, while ORF2 encodes a unique structural protein, the capsid protein, that plays important roles in viral packaging and immunogenicity [16, 17]. The capsid protein of approximately 90 kDa consists of a conserved N-terminal region (Cap-N) and a highly variable C-terminal region (Cap-C), forming the capsid shell and the capsid surface spike, respectively [18, 19]. The capsid polyprotein folds into the basic domain (~80aa), the inner core (S, ~180aa), the outer core (P1, ~150aa), the spike (P2, ~200aa) and the acidic domain (~140aa) [20].

Monoclonal antibodies (mAbs) are widely used in various biotechnological applications, including enzyme-linked immunosorbent assays, immunohistochemistry, flow cytometry, Western blotting, immunofluorescence assays, and vaccine design, owing to their high specificity, purity, and uniformity [21–24]. Previous studies have identified several B-cell epitopes of the capsid protein of GoAstV-2, and several detection methods based on mAbs have been developed [25–27]. These identified antigenic epitopes were focused on the variable C-terminal domain. However, the linear B-cell epitopes of the S domain of Cap-N have yet to be developed.

In this study, a recombinant truncated capsid protein of GoAstV-2 was expressed in *Escherichia coli* and purified using Ni–NTA His-Bind Resin. A mAb named 7B2 against the GoAstV-2 cap protein was prepared using hybridoma technology. A novel, highly conserved linear B-cell epitope was characterized by a series of truncated segments and synthetic peptides using indirect ELISA and Western blotting, and the results will aid further research on the B-cell epitopes of GoAstV-2.

Results

Expression and identification of the GoAstV-2 recombinant rCap protein

The recombinant plasmid pCold I-rCap was successfully expressed in *E. coli Transetta* (DE3) cells and purified from the supernatant post-sonication using a Ni–NTA affinity chromatography column. The molecular weight of the purified protein was approximately 29 kDa (Fig. 1A). The Western blot results (Fig. 1B) indicated that the recombinant rCap protein could react with anti-His mAb at a dilution of 1:2000.

Generation and characterization of the anti-rCap mAb

The purified rCap protein as an immunogen was injected into BALB/c mice, and hybridoma cells were produced using hybridoma technology. The cells were screened using indirect ELISA. The 7B2 hybridomas were ultimately selected for further analysis. The purified mAb titer analyzed using indirect ELISA was approximately 1:25,600. The Western blot results demonstrated that mAb 7B2 had a strong binding affinity with the denatured rCap protein (Fig. 2A). Immunofluorescence assay (IFA) was performed to evaluate the reaction between mAb 7B2 and the natural Cap protein. The mAb 7B2 and

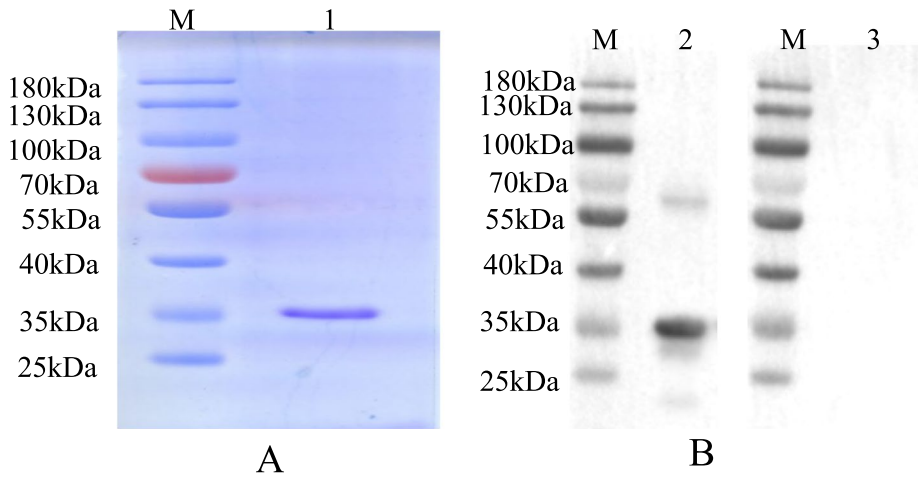


Fig. 1 SDS-PAGE and Western blot analysis of the purified pCold I-rCap. **A** SDS-PAGE. Lane M: protein marker; Lane 1: the purified pCold I-rCap protein. **B** Western blot analysis with anti-His mAb. Lane M: protein marker; Lane 2: the purified pCold I-rCap protein sample; and Lane 3: negative control

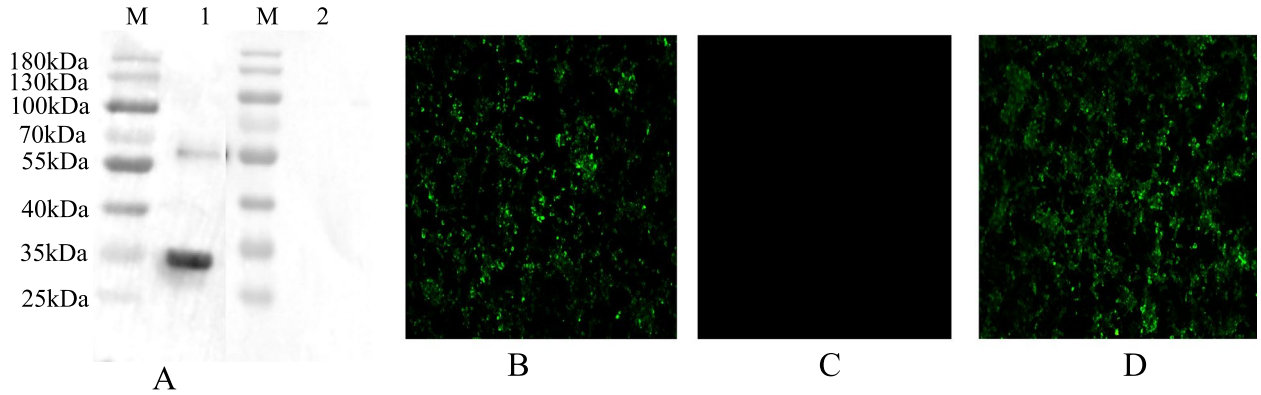


Fig. 2 Identification of the mAb 7B2 anti-GoAstV strain using Western blots and an IFA assay. **A** Western blot analysis. Lane M: marker proteins; Lane 1: the purified pCold I-rCap protein with mAb 7B2 as the primary antibody; and Lane 2: the purified pCold I-rCap protein with negative serum as the primary antibody. **B**-**D** IFA analysis of the LMH cells infected with GoAstV-2. **B** mAb 7B2; **C** negative serum; and **D** positive serum of GoAstV-2

positive serum were used as primary antibodies. In the IFA results (Fig. 2B-D), strong green fluorescence was observed in the LMH cells infected with GoAstV-2, indicating that mAb 7B2 could recognize the native Cap protein in infected cells.

The precise location of the 7B2 epitope on the Cap protein

Epitope mapping was performed based on the B-cell epitope prediction using the IEDB online analysis software (Fig. 3A). Three truncated segments that overlapped by 10aa, C1 (1-80aa), C2 (70-140aa), and C3 (130-210aa) were constructed into the vector pCold-MBP with His-tag and expressed in *E. coli Transetta* (DE3), and the recombinant pCold I-rCap protein (1-210aa) was used as a positive control. The western blot results showed that only the C3 segment could react with mAb 7B2, while the other two segments did not react (Fig. 3B), indicating that the potential epitope was located in the 130-210aa region of the Cap protein. The results were verified using indirect ELISA and were consistent with western blot results (Fig. 3C). During the second screening, the C3 segment was divided into two parts: C3-1 (130-176aa) and C3-2 (163-210aa). The results of western blot and indirect ELISA demonstrated that the C3-1 (130-176aa) fragment could be recognized by mAb 7B2, but the C3-2 (163–210) fragment could not (Fig. 3D and E). The C3-1 fragment was further truncated into three overlapping short peptides: C3-1a (130-153aa), C3-1b (149-165aa), and C3-1c (143-176aa). The mAb 7B only reacted with the C3-1b fragment as seen in the results of western blot and indirect ELISA (Fig. 3F and G), indicating that the antigenic epitope was located between aa 149 and aa 165 of the cap protein.

To more precisely identify the minimum linear epitope recognized by mAb 7B2, during the fourth and fifth rounds of truncation, eleven shortened peptides were synthesized by single amino-acid deletions from either the N-terminus or the C-terminus in the peptide sequence ^{149}EQEANTAGPESIDTIKA^{165}. The results using western blot and indirect ELISA assay are summarized in Fig. 4H-K. In addition to the peptides C3-1b-5, C3-1b-6, C3-1b-44, C3-1b-45, seven other peptides (C3-1b-1, C3-1b-2, C3-1b-3, C3-1b-4, C3-1b-41, C3-1b-42 and C3-1b-43) were recognized by mAb 7B2, whereas the C3-1b-5 peptide only weakly reacted with mAb 7B2, demonstrating that the minimum linear epitope of mAb 7B2 was ^{153}NTAGPESIDT^{162}.

Sequence alignment analysis of the identified antigenic epitope

To investigate the conservation of the identified linear B-cell epitope, the epitope regions of GoAstV-2 were compared with the corresponding areas of other species of astroviruses. The sequence alignment results revealed that the linear epitope ^{153}NTAGPESIDT^{162} was highly conserved, as it shared 100% sequence identity among the GoAstV-2 strains analyzed and showed 90% homology with a Turkey astrovirus 2 strain (Fig. 4). However, the GoAstV-1 and other astroviruses showed very low sequence homology (less than 50%) with the GoAstV-2 virus in the same region of the epitope, indicating that the identified linear epitope of mAb 7B2 was highly specific and conserved in the Cap protein of GoAstV-2.

Discussion

GoAstV-2 is a novel and acute infectious disease in goslings characterized by clinical symptoms of growth retardation, diarrhea, and death, and necropsy generally shows severe urate deposition in the joint cavities and

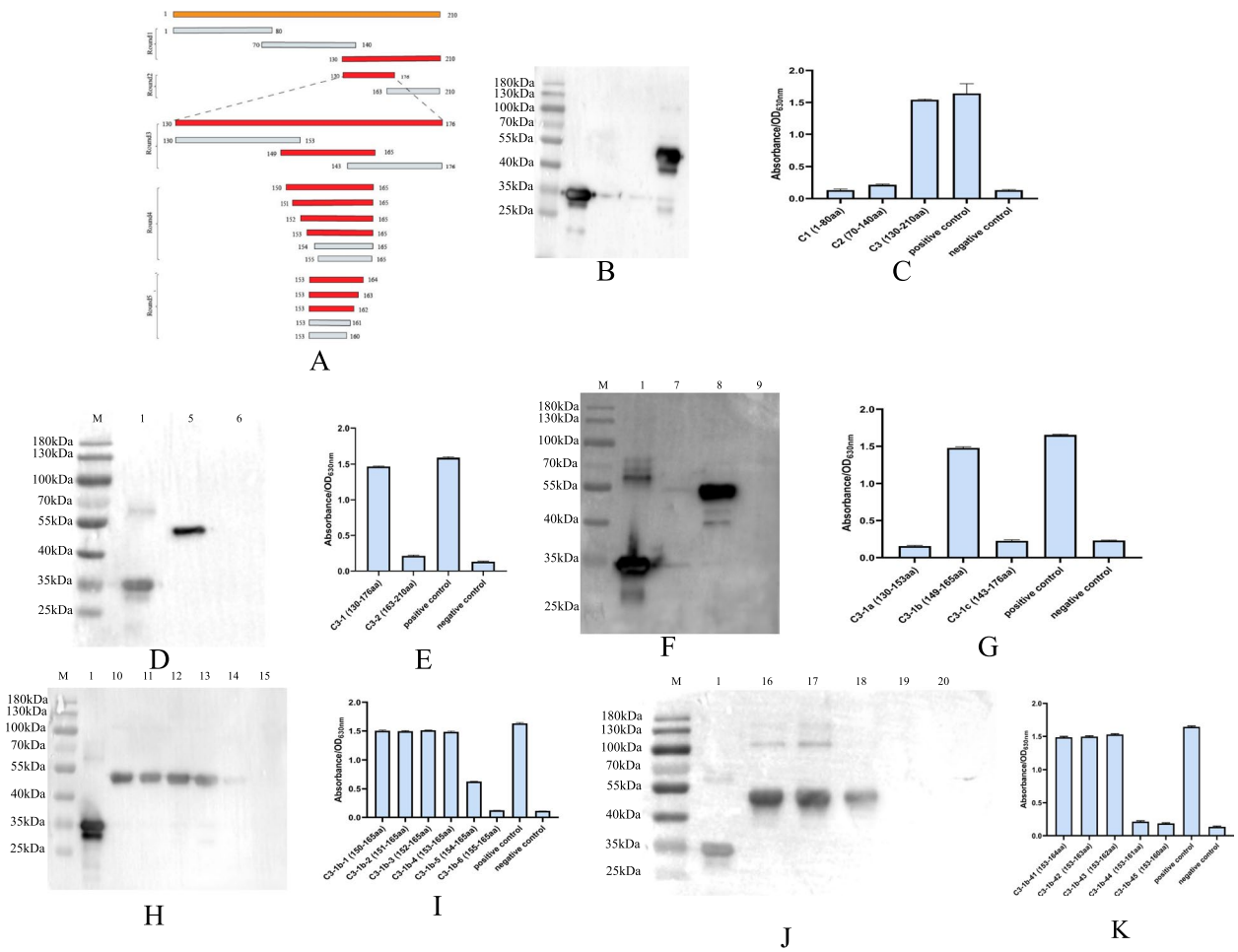


Fig. 3 Epitope indentification of the GoAstV-2 rCap protein. **A** Schematic of GoAstV-2 rCap protein epitope mapping. The segments marked in red were recognized by mAb 7B2, and those marked in gray were not recognized by mAb 7B2. **B**-**K** The reactivity of mAb 7B2 with a series of truncated rCap proteins using western blot and indirect ELISA assays. 2–20: C1, C2, C3, C3-1, C3-2, C3-1a, C3-1b, C3-1c, C3-1b-1, C3-1b-2, C3-1b-3, C3-1b-4, C3-1b-5, C3-1b-6, C3-1b-41, C3-1b-42, C3-1b-43, C3-1b-44, and C3-1b-45, respectively; 1: purified pCold I-rCap protein

multiple internal organs along with significant swelling and hemorrhage in the kidneys [28]. Zhang et al. first isolated the GoAstV-2 virus from LMH cells and named the strain GoAstV GD. The pathology was replicated with similar symptoms in an animal regression experiment in 2018 [14]. The virus can be transmitted between different animal species such as chickens and ducks, and outbreaks have caused significant economic losses for the waterfowl-breeding industry [29–31]. It is essential to continue to supervise and control GoAstV-2 to prevent the spread of the virus.

The capsid protein located at the 3' end of the genome undergoes a series of cleavages by trypsin proteases to produce VP34 (the N-terminal conserved region), VP27, and VP25 (the C-terminal variable region) [32]. The VP27 protein plays key roles in receptor recognition, virus attachment, and entry, and it can induce the host to generate neutralizing antibodies [33]. It has been reported that mAbs against VP27 protein with high sensitivity, specificity, and strong reactivity were obtained [26, 34, 35]. VP34 constitutes the capsid core region of the astrovirus and is highly hydrophilic, with a high antigenic index [36]. Compared to the VP27 protein, the N-terminal domain of the capsid protein is higher conservation and greater abundance in viral particles. These properties strongly support its prioritization as a target antigen for serological diagnostic methods [37]. In the present study, the N-terminus of GoAstV-2 Cap (rCap) protein was expressed, purified, and then used to inject BALB/c mice. A novel mAb against rCap protein was obtained and identified using IFA and Western blotting assays. The results showed that the mAb could specifically recognize GoAstV-2 in infected LMH and strongly react with the natural Cap protein, indicating that the mAb recognized conformational epitopes. The mAb titer was up to 1:25,600. Thus, the generated mAb in this study

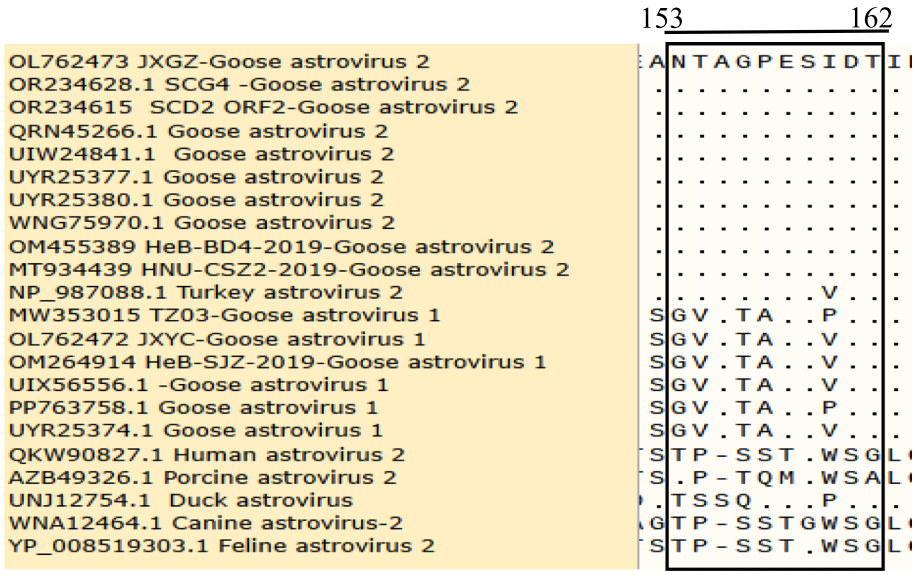


Fig. 4 Sequence alignment of 10 GoAstV-2 strains and 12 other astrovirus strains in the epitope region of the Cap protein. The amino acids from different astrovirus cap proteins are aligned. The matched amino acid residues are indicated by "", and "-" represents the absence of amino acids at this position

will help develop specific and sensitive diagnostic kits for detecting GoAstV-2 infection, using methods such as ELISA, immunochromatographic strips, and IHC, and the results will aid in further understanding the antigen–antibody interactions.

Identification of linear epitopes can play an important role in epitope-based vaccine design, protein structure analysis, diagnosis, and the development of therapeutic kits [38–40]. To date, only one linear B-cell epitope, ^{33}QKVY^{36}, has been identified in the basic domain of Cap-N [25]. However, knowledge of the function and structure of the GoAstV-2 Cap protein remains limited, and the mechanism underlying GoAstVs causing gout is unclear. In this study, 19 different overlapping truncated fragments were expressed in *E.coli* strain *Transetta* and analyzed using indirect ELISA and western blot assays for precise epitope mapping of mAb 7B2. The results revealed that the minimal epitope of mAb 7B2 was ^{153}NTAGPESIDT^{162}. The length of the identified epitope was less than 20 amino acids, consistent with a previous report [41].

The sequence alignment analysis demonstrated that the 7B2 epitope sequences were 100% identical among GoAstV-2 strains and had low similarity with GoAstV-1 as well as other non-goose astroviruses, except for Turkey astrovirus 2, indicating that it could be an optional target for the development of epitope-based diagnostic methods for GoAstV-2. Accroding to the results, only one amino acid substitution was found between GoAstV-2 and Turkey astrovirus 2, therefore, cross-reaction may be found with Turkey astrovirus 2, In that case, the mAb 7B2 may be used for Turkey identification. In a word, more works shall be done for further research.

Conclusion

In summary, a novel mAb (7B2) against the GoAstV-2 Cap protein was successfully obtained, and the minimal linear B-cell epitope was identified as ^{153}NTAGPESIDT^{162} located in the N-terminal region. This epitope showed high sensitivity and specificity as well as being highly conserved among 10 representative GoAstV-2 strains. The findings will be useful for further studying the structure and function of the GoAstV-2 Cap protein, and the results provide a potential candidate target for the establishment of a specific and sensitive diagnostic method for controlling and monitoring the spread of GoAstV-2.

Materials and methods

Virus, cells, animals and plasmids

The GoAstV-2 strain JX01 (GenBank Accession No. MZ576222) [42, 43] and the Leghorn Male Hepatoma (LMH) cell line were stored in our laboratory. The SP2/0 cells were preserved at the Guangdong Laboratory Animals Monitoring Institute. The cells were maintained in Dulbecco's modified Eagle medium (Wisent, Nanjing, China) supplemented with 10% fetal bovine serum (FBS) (Wisent, Nanjing, China) and 2% penicillin–streptomycin (Solarbio, Beijing, China) at 37 °C in a humidified 5% CO_2 atmosphere. Six- to eight-week-old female

BALB/c mice were obtained from the Guangdong Medical Laboratory Animal Center (Guangdong, China). The truncated ORF2 gene sequence (nucleotides 1–630 bp) of the GoAstV-2 strain was synthesized by Gene Create (Wuhan, China) and cloned into a pCold I expression vector to construct the recombinant plasmid pCold I-rCap, and the plasmid was identified through PCR and sequencing.

Expression and purification of recombinant GoAstV-2 rCap protein

The recombinant plasmid pCold I-rCap was transformed into *Escherichia coli Transetta* (DE3) (TransGen Biotech, Beijing, China) for protein expression induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 16 °C. The expressed recombinant GoAstV-2 rCap protein was confirmed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and purified using Ni–NTA His-Bind Resin (Qiagen, Germany) according to the manufacturer's instructions. The purified protein was evaluated using SDS-PAGE and Western blots with the positive GoAstV-2 serum. The concentration of the total protein was calculated with a BCA protein assay kit (Beyotime, Shanghai, China).

Preparation of anti-cap protein monoclonal antibodies

To generate the mAbs anti-rCap protein, five 6–8-week-old female BALB/c mice were inoculated by subcutaneous multi-point injection with purified recombinant rCap protein (100 μg per mouse) mixed with Freund's complete adjuvant (Sigma, USA) as the initial immunization. The mice were subsequently boosted with 100 μg of antigen emulsified in Freund's incomplete adjuvant (Sigma, USA) at 14 and 28 days. After the third immunization, the titers of serum antibody were tested by indirect ELISA as described in the following text. Once the titers reached 1:100,000, a final boost was performed with 50 μg of protein without the adjuvant 3 days before fusion. Splenocytes obtained from the immunized mice were fused with SP2/0 cells using polyethylene glycol 1500 (Sigma, USA), and hybridoma cells were screened in HAT and HT medium with 15% FBS following a standard protocol [44]. The culture supernatants of hybridoma cells were measured using indirect ELISA. To generate stable antibody-secreting hybrid cell lines, the positive hybridomas were subcloned four times using the limited dilution method. Five days after the mice were prestimulated with 500 μL of Freund's incomplete adjuvant, 500 μL of hybridoma cells containing 1×10^6 cells was intraperitoneally injected into the mice to obtain ascites fluid. The mAb from the ascites fluid was harvested using HiTrap® Protein G prepacked columns [45].

Indirect enzyme-linked immunosorbent assay

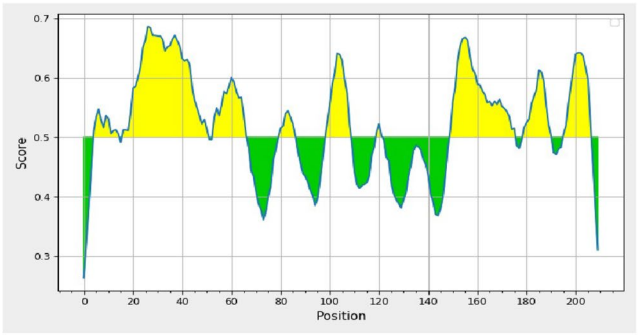
An indirect ELISA was developed to analyze the titers of serum antibodies and mAbs in this study. The indirect ELISA was performed as previously described with several modifications [46]. Briefly, a 96-well plate was coated with 0.5 μg/well antigen at 37 °C for 2 h. The plate was washed five times with phosphate-buffered saline containing 0.05% Tween-20 (PBST) and blocked with 5% skim milk (BD Biosciences, USA) in PBS for 2 h at 37 °C. After washing five times and drying the plate, the primary antibody (100 μL/well) was added to the plate and incubated for 1 h at 37 °C. After incubation and five washes, a dilution of 1:2000 HRP-conjugated goat anti-mouse IgG (Beyotime, China) was added to each well, and the plate was incubated at 37 °C for 1 h. Finally, 100 μL of substrate solution (Beyotime, China) was added to all wells, and plates were kept in the dark for 10 min. The absorbance value at 630 nm was read using a microplate reader (Thermo Fisher, USA).

Immunofluorescence assay

To identify the GoAstV-2 in infected cells recognized by mAb 7B2, an immunofluorescence assay was performed as described previously [47]. Briefly, the LMH cells were seeded in 96-well cell plates, cultured to 80% density, and then infected with GoAstV-2 strain JX01 (MOI = 0.01). At 12 h post-infection, the cells were fixed with 70% precooled ethanol for 30 min at 4 °C, then washed three times with PBST, and blocked with 1% bovine serum albumin (Sigma, USA) in PBST for 1 h at 37 °C. The primary antibody at a dilution of 1:5000 was added to the plate and inoculated at 37 °C for 1 h, followed by washing three times with PBST. The plate was inoculated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Thermo Fisher, USA) used as secondary antibody (1:500 dilution) at 37 °C for 1 h in the dark. Finally, the cells were observed under a fluorescence microscope (Leica Microsystems, Germany).

Western blot

Bacteria expressing the proteins were centrifuged at $12{,}000\times g$ for 5 min to collect the precipitant as the sample. The samples were mixed with 5 × loading buffer, boiled for 10 min at 100 °C, and then subjected to 12% SDS-PAGE to separate the proteins. The proteins were then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, USA). The membrane was blocked with 5% skim milk in PBST for 2 h at room temperature (RT). After washing five times with PBST, the membrane was inoculated with 1:5000 diluted mAb for 2 h at RT, then rinsed five times with PBST, and finally reacted with HRP-conjugated goat



No.	Start	End	Peptide	Length
1	5	15	AVAPREKVTKK	11
2	17	51	TKVVTVKKKHPKKKPKQKVYKPQKLPMKAERKLEK	35
3	54	67	KGLKKRVAGPPVND	14
4	81	87	NSTDTLD	7
5	100	109	KNQENGQTAT	10
6	120	122	LWR	3
7	150	176	QEANTAGPESIDTIKARPHLELPIGSK	27
8	180	191	RVQPRLMQGPRQ	12
9	196	207	VDPGDSPTDSLG	12

B

Fig. 5 B-cell epitope prediction results for the GoAstV-2 rCap protein using the IEDB online analysis software. **A** The yellow color represents the antigen epitope. **B** The amino acid sequences of the predicted peptides

Table 1 Primer sequences used for linear epitope identification

Fragments	Primer sequences (5′–3′)		Position (bp)
	Sense	Reverse sense	
C1	CC*CTCGAC*ATGGCAGACAGGGCGG	CG*GAATTC*CCCAGTGATCTGACCAAGTGT	1–240
C2	CC*CTCGAC*ACTACCACAATAACACTTGG	CG*GAATTC*AAGAATATTGGCCCTACCAGCAAG	208–420
C3	CC*CTCGAC*CTTGTCCCACTTGCTGGTAGG	CG*GAATTC*AATTGCTGGACCAAGTGAGTC	388–630
C3-1	CC*CTCGAC*CTTGTCCCACTTGCTGGTAGG	CG*GAATTC*TTTTGAGCCAATCGGGAGTT	388–528
C3-2	CC*CTCGAC*ATAAAGGCTCGGCCGCATCT	CG*GAATTC*AATTGCTGGACCAAGTGAGTCAG	487–630

The restriction enzyme sites of the sense and reverse sense are *BamH I* and *EcoR I* (underlined), respectively

anti-mouse IgG as a secondary antibody for 1 h at RT. The results were analyzed using the Azure c600 multi-functional molecular imaging system (Azure Biosystems, USA).

Identification of the truncated recombinant rCap protein and the minimal antigenic epitope

The linear B cell epitopes of the GoAstV-2 rCap protein (1-210aa) were predicted using the IEDB bioinformatic analysis website (https://www.iedb.org/) based on the amino acid sequence obtained from NCBI GenBank (ID: OL762473.1). To accurately locate the minimal epitope recognized by mAb 7B2, six rounds of truncation were performed according to the prediction results (Fig. 5). A set of primers for amplifying the truncated gene fragments and polypeptides was designed and synthesized by Sangon Biotech. The sequences of primers are listed in Table 1, and the polypeptides are listed in Table 2. These truncated overlapping fragments and polypeptides were cloned into the pCold I-MBP vector and expressed in *E. coli Transetta* (DE3) as described previously. The fusion proteins were analyzed using Western blots and indirect ELISA with mAb 7B2.

Table 2 Sequences of the synthesized polypeptides used in this study

Name	Amino acid sequences in the cap protein position
C3-1a	^{130}LVPLAGRANILGSVVFLDIEQEAN^{153}
C3-1b	^{149}EQEANTAGPESIDTIKA^{165}
C3-1c	^{160}IDTIKARPHLELPIGSK^{176}
C3-1b-1	^{150}QEANTAGPESIDTIKA^{165}
C3-1b-2	^{151}EANTAGPESIDTIKA^{165}
C3-1b-3	^{152}ANTAGPESIDTIKA^{165}
C3-1b-4	^{153}NTAGPESIDTIKA^{165}
C3-1b-5	^{154}TAGPESIDTIKA^{165}
C3-1b-6	^{155}AGPESIDTIKA^{165}
C3-1b-41	^{153}NTAGPESIDTIK^{164}
C3-1b-42	^{153}NTAGPESIDTI^{163}
C3-1b-43	^{153}NTAGPESIDT^{162}
C3-1b-44	^{153}NTAGPESID^{161}
C3-1b-45	^{153}NTAGPESI^{160}

Sequence alignment analysis of the identified antigenic epitopes

The amino acid sequences of 10 prevalent GoAstV-2

strains and 12 other related astrovirus strains, including GoAstV-1, porcine astrovirus 2 strain, canine astrovirus 2 strain, human astrovirus 2 strain, turkey astrovirus 2 strain, feline astrovirus 2 strain, and duck astrovirus strain, were downloaded from the NCBI GenBank database and aligned using SnapGene software to analyze the conservation of the identified antigenic epitope.

Abbreviations

GoAstV-2	Goose astrovirus genotypes 2
mAb	Monoclonal antibody
GoAstV-1	Goose astrovirus genotypes 1
IFA	Immunofluorescence assay
WB	Western blot
ELISA	Enzyme-linked immunosorbent assay

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04687-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Authors' contributions

LHQ and ZYJ wrote the main manscript text. HHY and ZYB revised the manscript. CL and TJ prepared figures 1-2. LN, ZFF and WCC prepared figures 3-5. HJN, WQP and WSY prepared table 1-2 and edited the manscript. All authors reviewed the manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Jiangxi Agricultural University (Approval ID: JXAULL-2017003). All operations were carried out in accordance with the Sanitary Standard for the Non-hazardous Treatment of Might Soil (GB7959-87).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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