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A simple and rapid method for generating antibodies against bovine alphaherpesvirus 1 viral proteins through immunization with virus-infected murine cells

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ABSTRACT

Bovine alphaherpesvirus 1 (BoAHV-1) infects cattle and typically results in significant economic losses for the cattle industry worldwide. Currently, antibodies targeting only a limited number of viral proteins are commercially available. It has been reported that BoAHV-1 is capable of infecting numerous tumor cell lines. Based on the rationale of immune tolerance, we hypothesized that virus-infected murine cells could be directly used to immunize mice, thereby generating antibodies against viral proteins. In this study, we found that BoAHV-1 can infect murine cell lines including LA795 and MC38, as determined using both Western blot and immunofluorescence analyses. Immunizing mice with virus-infected cells, either through subcutaneous or intraperitoneal injection, stimulates the production of high levels of antibodies that specifically recognize the viral proteins synthesized in bovine kidney (MDBK) cells, as characterized by both Western blot and/or immunofluorescence. Furthermore, our findings suggest that intraperitoneal immunization could more effectively elicit antibodies against a wider array of viral proteins. As a homemade antibody generation method, this approach bypasses the complex and time-consuming steps of producing and purifying recombinant proteins as antigen, which are typically performed in conventional methods for antibody generation. Thus, we present a simple, rapid, and cost-effective method for generating virus-specific antibodies.

1. Introduction

Bovine alphaherpesvirus 1 (BoAHV-1), is a member of the genus *Varicellovirus* in the subfamily Alphaherpesvirinae under the family Herpesviridae. It is an important viral pathogen, associated with a variety of inflammatory diseases in various systems, such as the upper respiratory and genital tract [1–3]. The viral genome comprises a 106 kb unique long segment (UL) and a 10 kb unique short region (US), which is flanked by inverted repeats of 11 kb [4]. This genome encodes at least 73 open reading frames (ORFs) that have been identified [5,6]. For example, at least 12 glycoproteins of the alpha herpesvirus have been identified, including gB, gC, gD, gE, gH, gI, gG, gK, gL, gM, gN, and gJ [7–9], which are potentially involved in virus infection and pathogenicity. During BoAHV-1 productive infection, vial genes are expressed in a temporally regulated cascade and are classified into three kinetic classes: immediate early (IE), early (E), or late (L) [10,11].

As we known, antibodies are essential for investigating the viral replication mechanism both *in vitro* and *in vivo*. However, only a limited number of commercial antibodies against a few viral proteins are available, such as gC and gD, and some homemade antibodies against viral IE or E proteins, such as bICP0, bICP4, bICP22, and VP16 have been used for extensive studies [12–15]. While these antibodies have greatly contributed to our understanding of the virus infection mechanism, more antibodies targeting a wider range of viral proteins are required to support further investigations into the virus pathogenicity.

As a potential oncolytic virus, BoAHV-1 is capable of infecting a

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broad spectrum of immortalized or transformed human tumor cells while sparing of healthy cells [16,17]. Additionally, it can infect the mouse neuroblastoma cell line (Neuro-2A), albeit with lower efficiency [18–21], which is reminiscent of the utilization of virus-infected murine cells as an antigen carriers to immunize mice for the generation of antibodies against viral proteins. The rationale is that mice will exhibit minimal immune responses to introduced cellular proteins of murine origin due to immune tolerance to these self-proteins, but will mount a robust immune response to viral proteins.

Here, we found that both murine cell lines LA795 and MC38 are permissive for BoAHV-1 replication. Furthermore, by immunizing mice with virus-infected LA795 and MC38 cells, we successfully generated antibodies specific to viral proteins, offering a simple method for generating viral protein-specific antibodies.

2. Material and methods

2.1. Virus and cells

Murine cell lines LA795 and MC38, and bovine kidney (MDBK) cells were purchased from the Chinese Model Culture Preservation Center in Shanghai, China. They were routinely passaged and maintained in either Dulbecco's Modified Eagle Medium (DMEM) or RPMI 1640 medium, supplemented with 10 % fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT, USA). BoAHV-1 strain NJ-16-1 was isolated from commercial bovine semen [22]. The virus was propagated in monolayer MDBK cells in 100 mm dishes, aliquoted (in a volume of 100 μL) and stored at −80 °C for future use.

2.2. Antibodies

BoAHV-1 gD mAb (VMRD, cat#1B8–F11) and goat anti-BoAHV-1 serum (cat# PAB-IBR) generated by immunization of goat with purified BoAHV-1 viral particles was ordered from VMDR Inc (Pullman, WA, USA). HRP- (horseradish peroxidase-) conjugated goat anti-mouse IgG (cat# BF03001) was purchased from Biodragon Technology (Suzhou, China). HRP- conjugated donkey anti-goat IgG (cat# ab97110) and Alexa Fluor 488-conjugated donkey anti-goat IgG (cat# ab150129) were provided by Abcam (Cambridge, UK). Alexa Fluor 633-conjugated goat pAb to mouse IgG (cat# A-21052) was provided by Invitrogen Life Technologies (Waltham, MA, USA).

2.3. Western blot

Cell lysates were separated on 8 % SDS-PAGE gels and then transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). After blocking with 5 % non-fat milk in PBST for 1 hour (h) at room temperature, the membranes were incubated with indicated primary antibodies overnight at 4°C. Then they were incubated with secondary antibodies, such as HRP-conjugated goat anti-mouse IgG (cat# BF03001, dilution 1:10000) or HRP- conjugated donkey anti-goat IgG (cat# ab97110, dilution 1:10000) for 1 h at room temperature. After extensive washing with PBST, the reactive protein bands were developed using Clarity Western ECL substrate (Bio-Rad, Hercules, CA, USA, cat#1705061).

2.4. Immunization of mice

Animals care and study procedure were following the guideline of the Animal Research Ethics Board of Hebei University (Approval number 402,106,002) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council. Mice were purchased from Company of Charles River (Beijing, China). One group of Balb/c female mice (n = 3) at six-week old were anesthetized with inhaled isoflurane (4 % induction and 2 % maintenance), then immunized via intraperitoneal injection with 1×10^7 of virus-infected LA795 cells in a volume of 100 μL. After two weeks, they received a booster immunization with 1×10^7 of virus-infected LA795 cells via intraperitoneal injection. The second and third groups of Balb/c female mice (n = 3) at six-week old were anesthetized with inhaled isoflurane (4 % induction and 2 % maintenance), then immunized via subcutaneously injections with 1×10^7 of virus-infected LA795 and MC38 cells emulsified in complete Freund's adjuvant(Sigma-Aldrich, St. Louis, MO, USA) (dilution volume of 1:1) in a volume of 100 μL, respectively. After two weeks, they received a booster immunization subcutaneously with 1×10^7 of virus-infected LA795 and MC38 cells emulsified in incomplete Freund's adjuvant (in a volume of 100 μL). At four weeks post the second booster, the animals were humanely euthanized via CO2 inhalation for the collection of sera for subsequent studies. Prior to the final booster immunization, approximately 20 μL of blood was collected from the tail end for subsequent identification using Western blot analysis. Of note, as an oncolytic virus, BoAHV-1 is capable of reducing the viability of infected cells. To further reduce the risk of oncogenicity of these tumor cells, they were exposed to UV-irradiation prior to intraperitoneal injection, as previously reported [23].

2.5. Immunofluorescence assay (IFA)

LA795, MC38, and MDBK cells in 24-well chamber slides (Nunc Inc., IL, USA) were either mock infected or infected with BoAHV-1 at an MOI of 1. After infection for 24 h or 48 h, cells were fixed with 4 % paraformaldehyde in PBS for 10 min at room temperature, permeabilized with 0.25 % Triton X-100 in PBS for 10 min at room temperature, and blocked with 1 % BSA in PBST for 1 h followed by incubation with goat anti-BoAHV-1 serum (cat# PAB-IBR, 1:1000) or the mouse sera (1:500) generated by this study in 1 % BSA in PBST overnight at 4 °C. After three washes with PBS cells were incubated with Alexa Fluor 488-conjugated donkey anti-goat IgG (cat# ab15012, 1:2000) or Alexa Fluor 633-conjugated goat pAb to mouse IgG (cat# A-21052, 1:2000) for 1 h in the dark at room temperature. After three additional washes, the nuclei were stained using DAPI (4′,6-diamidino-2-phenylindole). Slides were covered with coverslips by using antifade mounting medium (Electron Microscopy Sciences, cat# 50-247-04). Images were captured using a confocal microscope (Zeiss). Quantitative analysis of the fluorescence intensity was performed using the Image J program, a free software available for download from the provided link (https://imagej.nih.gov/ij/download.html). The analysis was performed following the conventional protocol for the software usage.

3. Results

3.1. BoAHV-1 infects murine cell lines LA795

The tumor cell line LA795, derived from mouse lung adenocarcinoma[24], has not been previously reported to be permissive to BoAHV-1 infection. To assess the susceptibility of this cell line to viral infection, the cells were infected at a high multiplicity of infection (MOI) of 10 for 24 and 48 h, respectively. Notably, to achieve efficient infection, the cells were inoculated at a higher MOI of 10 and incubated for a longer duration than that used for MDBK cells, based on our preliminary studies (Fig. S1A). Under microscopic observation, typical cytopathic effects (CPE), including cell death and forming clusters, were induced after 48 h of infection (Fig. 1A). After infection for 24 and 36 h, cell lysates were prepared and subjected to Western blot analysis to detect the expression of viral proteins. As observed in virus-infected MDBK cells, gD protein with molecular weight of approximately 70 kDa [25], was detected in virus-infected LA795 cells at both 24 and 36 h post-infection (hpi) using a commercial gD-specific monoclonal antibody (mAb) (VMRD, cat#1B8–F11) (Fig. 1B). When the expression of virion-associated proteins was detected using a commercial goat anti-BoAHV-1 serum (VMDR, cat# PAB-IBR), which was generated by

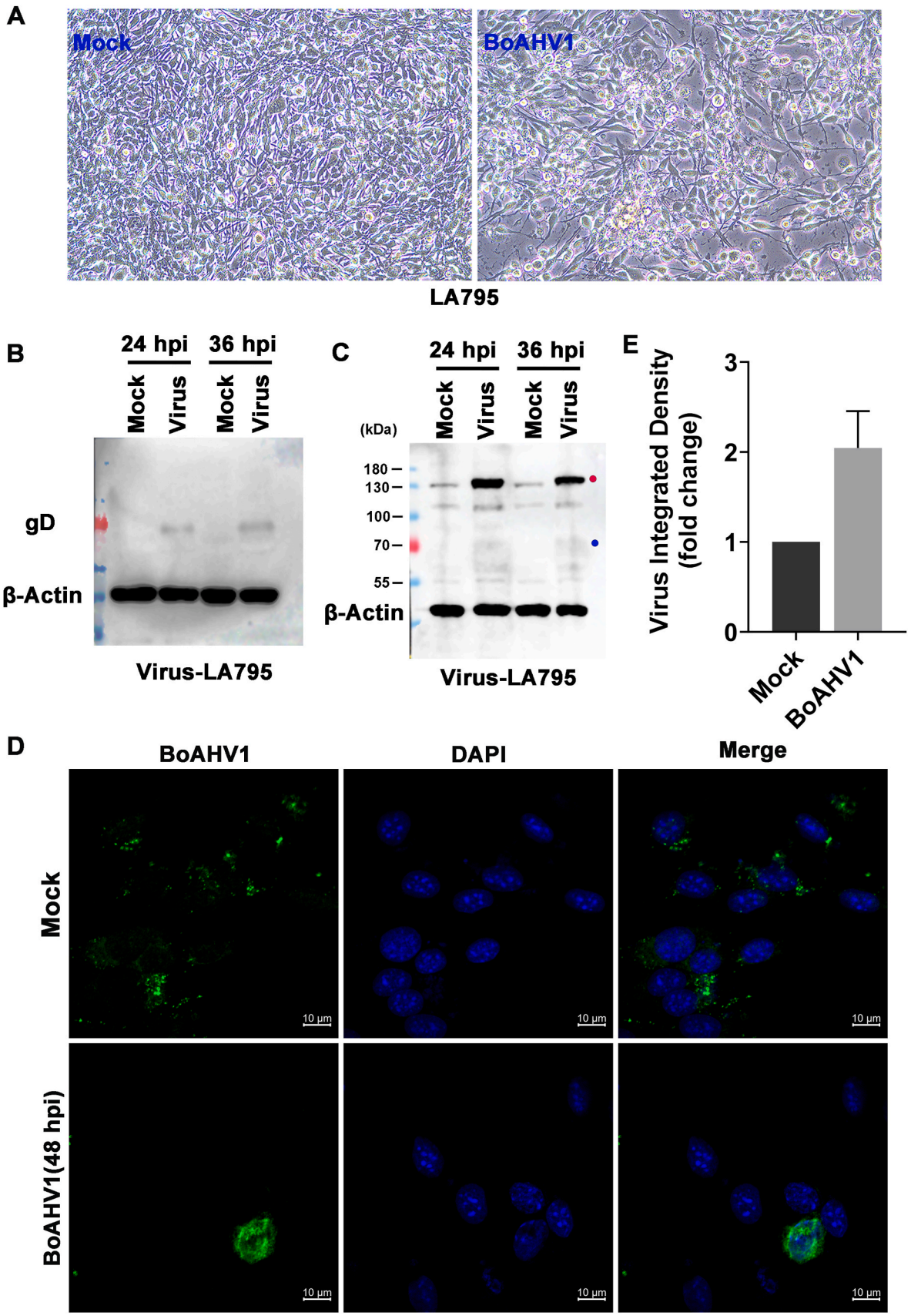

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Fig. 1. Detection of BoAHV-1 viral protein expression in virus infected LA795 cells.
(A) LA795 cells in 24-well plates were either mock-infected or infected with BoAHV-1 at an MOI of 10 for 48 h. Cell morphology was then observed under a light microscope. Images shown are representative of two independent experiments (magnification: 100 ×).
(B and C) LA795 cells in 60 mm dishes were either mock-infected or infected with BoAHV-1 at an MOI of 10 for 24 and 36 h, respectively. Subsequently, cell lysates were prepared and subjected to Western blot analysis to detect viral proteins using BoHV-1 gD mAb (VMRD, cat#1B8–F11, 1:2000) (B) and goat anti-BoAHV-1 serum (cat# PAB-IBR, 1:6000) (C), respectively. The protein ladders were provided by ThermoFisher (cat# 26616). Data shown are representative of two or three independent experiments.
(D) LA795 cells were either mock-infected or infected with BoAHV-1 (MOI = 10) for 48 h. Subsequently, the cells were immunostained via using an antibody against virion-associated proteins (cat# PAB-IBR, 1:1000), then visualized by confocal microscopy. Nuclei were stained with DAPI (blue). The images were captured using confocal microscopy. Images shown are representative of three independent experiments. Scale bar = 10 μm
(E) Fluorescence intensity was quantified with ImageJ and normalized to the mock-infected control, which is arbitrarily set to 1.

immunizing goats with purified BoAHV-1 particles, a major band with a molecular weight of approximately 130 kDa (denoted by a black-filled circle) was observed, showing intense staining, and a relatively faint band with a similar molecular weight was also detected in mock-infected cells, suggesting the antibody may have cross-reactivity with host proteins. Additionally, a faint, smear-like band at a molecular weight of approximately 70 kDa (denoted by blue-filled circles) was exclusively observed in virus-infected cells (Fig. 1C), indicating a specific reactivation with viral proteins. IFA analysis was conducted as an independent method to detect viral protein expression in LA795 cells using goat anti-BoAHV-1 serum (VMDR, cat# PAB-IBR). Consistent with the Western blot results, non-specific staining was observed in mock-infected cells (Fig. 1D, upper panels), and intensive staining was exclusively detected in virus-infected cells (Fig. 1D, bottom panels). Quantitative analysis indicated that the fluorescence intensity increased to approximately 2.04-fold following virus infection (Fig. 1E). These

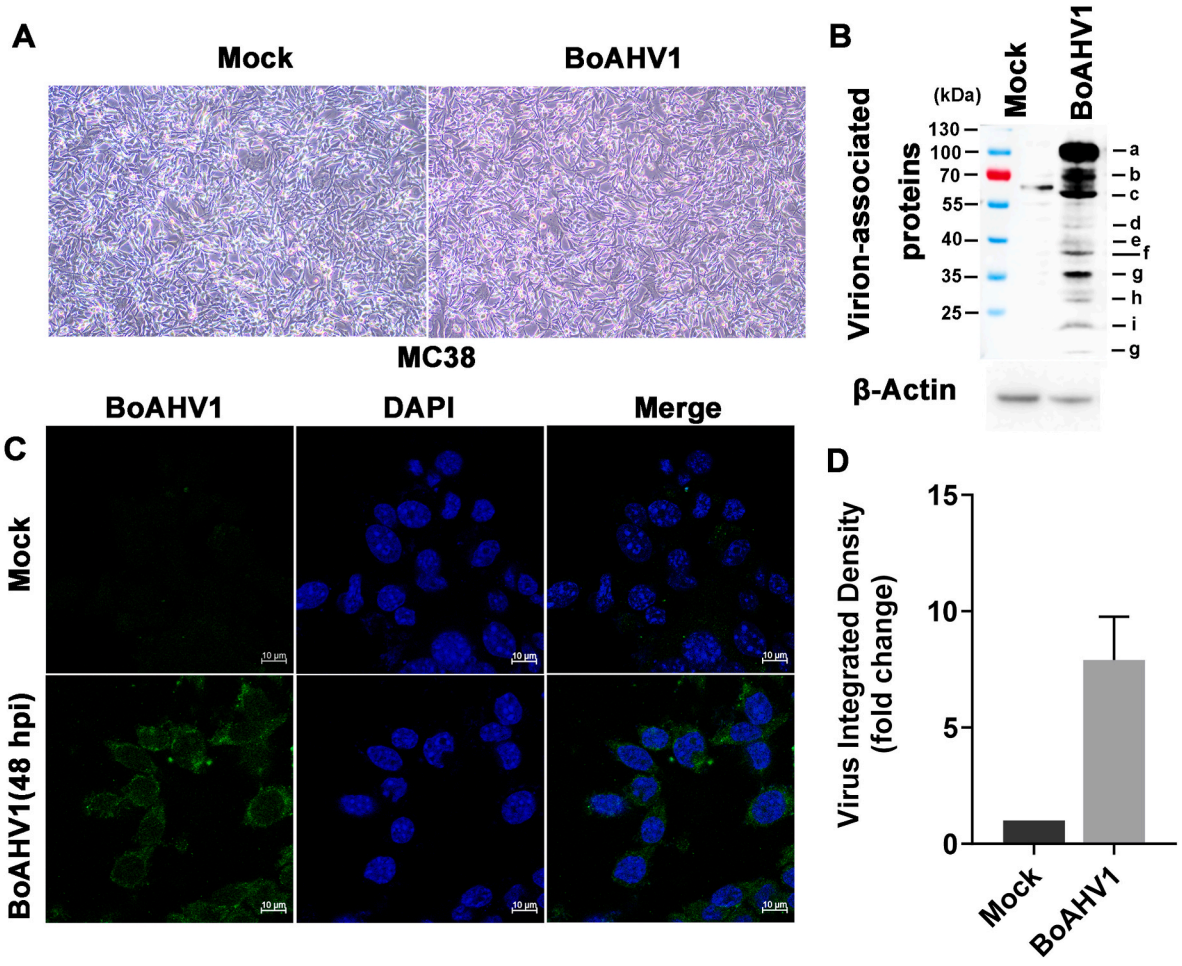

Fig. 2. Detection of BoAHV-1 viral protein expression in virus infected MC38 cells.
(A) MC38 cells were either mock-infected or infected with BoAHV-1 at an MOI of 10 for 48 h. Cell morphology was observed under a light microscope. Images shown are representative of two independent experiments (magnification: 100 ×).
(B) MC38 cells were either mock-infected or virus infected with BoAHV-1 at an MOI of 10 for 48 h. Subsequently, cell lysates were prepared and subjected to Western blot analysis to detect viral proteins using goat anti-BoAHV-1 serum (cat# PAB-IBR, 1:6000) The protein ladders were provided by ThermoFisher (cat# 26616). Data shown are representative of three independent experiments.
(C) After infection with BoAHV-1 (MOI = 10) for 48 h, MC38 cells were immunostained using an antibody against virion-associated proteins (cat# PAB-IBR, 1:1000) and then visualized by confocal microscopy. Nuclei were stained with DAPI (blue). Images were captured using confocal microscopy. Images shown are representative of three independent experiments. Scale bar = 10 μm
(D) Fluorescence intensity was quantified with ImageJ and normalized to the mock-infected control, which is arbitrarily set to 1.

data indicated that virion-associated proteins were specifically recognized by the viral antibody.

MC38 is a mouse colon adenocarcinoma cell line [26]. And also it has not been previously reported to be permissive to BoAHV-1 infection. When MC38 cells were infected with BoAHV-1 at an MOI of 10 for 48 h, typical CPE induced by the virus infection were not as prominently observed as in LA795 cells (Fig. 2A and Fig. S1B). The expression of virion-associated proteins was detected by Western blot using a commercial goat anti-BoAHV-1 serum (VMDR, cat# PAB-IBR). A major band with a molecular weight of approximately 100 kDa (denoted by the

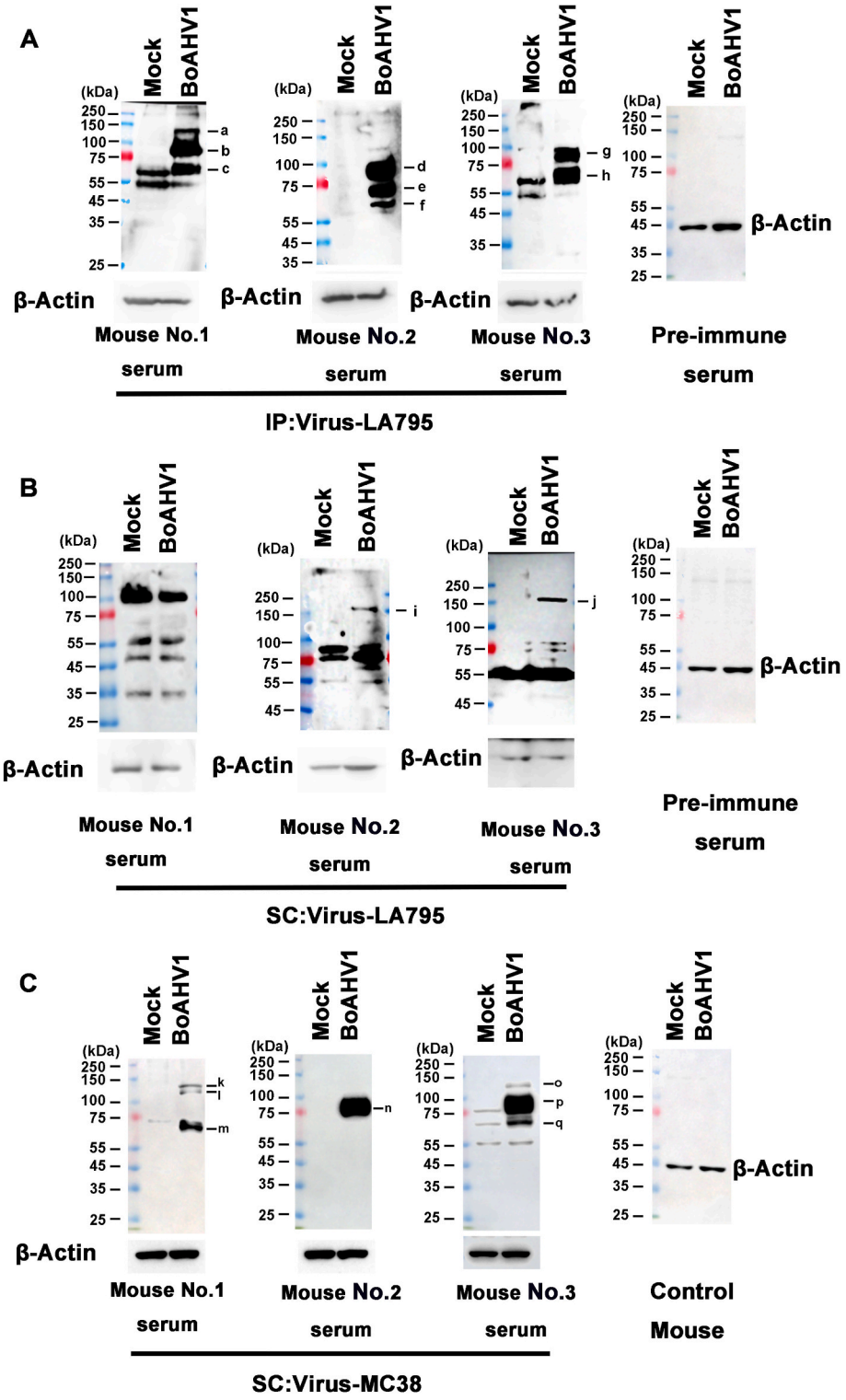

Fig. 3. Identification of the reactivation of mice sera prepared by immunization with virus-infected murine cell lines using Western blot.
MDBK cells were either mock infected or infected with BoAHV-1 at an MOI of 1 for the 24 h. Subsequently, cell lysates were prepared and subjected to Western blot analysis to detect viral proteins using the indicated mouse sera produced by immunization with LA795 cells via intraperitoneal injection (A), as well as LA795 (B) and MC38 (C) cells via subcutaneous injection, respectively. The protein ladders were provided by New Cell & Molecular Biotech (cat# P9006). The data shown are representative of three independent experiments.

letter “a"), showing intense staining, was observed. Additionally, seven other bands denoted by the letters “b" through “h" each showing varied immunostaining intensity, were exclusively observed in virus-infected cells. Non-specific reactivity with a panel of host proteins in mock-infected cells was also observed, but these did not overlap with the molecular weights of the viral proteins (Fig. 2B). With the IFA assay, we found that viral proteins could be detected in virus-infected MC38 cells but not in mock-infected cells (Fig. 2C), with fluorescence intensity increased to approximately 7.90-fold following virus infection, according to the quantitative analysis (Fig. 2D).

Thus, the two murine cell lines, including LA795 and MC38, support BoAHV-1 viral replication or, at a minimum, facilitate the complete expression of viral L genes, as evidenced by the detected virion-associated proteins.

3.2. Identification of antibodies in sera from mice immunized with virus-infected cell lines using Western blot analysis

Two groups of BALB/c mice (n = 3) were immunized with virus-infected LA795 cells via subcutaneous and intraperitoneal injections, respectively. A third group of mice (n = 3) received immunization with virus-infected MC38 cells through subcutaneous injection. As a control, one faint band with molecular weight ranging from 150 to 100 kDa were detected using serum from preimmune mice (Fig. 3A). All these three mice immunized with virus-infected LA795 cells via intraperitoneal injection produced antibodies specific to viral proteins (Fig. 3A). Specifically, serum from mouse No.1 recognized two viral proteins: “a” with molecular weights between 100 and 150 kDa, and “b” with molecular weights between 75 and 100 kDa. We are uncertain if band “c” is viral-specific, as a relatively faint band with an equivalent molecular weight was also observed in mock-infected cells (Fig. 3A, left panels). Serum from mouse No.2 recognized three viral proteins: “d” with molecular weights between 75 and 100 kDa, and “e” and “f” with molecular weights between 55 and 75 kDa (Fig. 3A, middle panels). Serum from mouse No.3 recognized one viral protein: “g” with molecular weights between 75 and 100 kDa. Although another band denoted by “h” showed intense staining, we are uncertain if it is viral-specific, as a relatively faint band with an equivalent molecular weight was also observed in mock-infected cells (Fig. 3A).

And serum from the preimmune mouse exhibited only non-specific recognition of one host protein from both mock-infected and virus-infected MDBK cells (Fig. 3A and B). Two of these three mice immunized with virus-infected LA795 cells via subcutaneous injection produced antibodies specific to a single viral protein. Serum from mouse No.1 did not specifically recognize any viral protein (Fig. 3B, left panels). One individual protein, denoted by “i” and “j” respectively, was recognized by mouse serum No.2 and No.3, each with a molecular weight of approximately 150 kDa (Fig. 3B).

All three mice immunized with virus-infected MC38 cells via subcutaneous injection produced antibodies specific to viral proteins. These proteins were denoted by “k”, “l”, and “m” and were recognized by mouse serum No.1; band “n” probed by mouse serum No.2, and three bands: “o”, “p”, and “q” detected by mouse serum No.3, respectively. Band “k” and “l” exhibits a molecular weight of approximately 100–150 kDa (Fig. 3C, left panels). Bands “m” and “n” exhibit molecular weights at below and above 75 kDa, respectively. Bands “o”, “p”, and “q” ranged from 55 to 150 kDa. The detailed information of these detected bands were summarized and shown in Table 1.

Taken together, immunization of mice with virus-infected cells of both LA795 and MC38 via either subcutaneous or intraperitoneal injections can induce the production of virus-specific antibodies. However, some mice may fail to produce these antibodies, and the antibodies that are produced may recognize different viral proteins, which may vary with different mice and immunization approaches.

3.3. Identification of antibodies in sera from mice immunized with virus-infected cell lines using immunofluorescence assay (IFA)

We conducted further analysis to determine whether the generated antibodies could be used to perform IFA analysis to detect viral proteins in cell cultures (MOI of 1, 24 hpi). Immunostaining was observed in virus-infected MDBK cells, exhibiting distinct profiles that varied with different mouse sera. In contrast, non-specific staining was rarely observed in mock-infected cells. These findings suggest that the generated antibodies, particularly those from mouse No.2, produced by immunizing mice with virus-infected LA795 cells via intraperitoneal injections, may be effective for detecting viral proteins through IFA analysis. However, serum from mice (No.3) subcutaneously immunized with virus-infected MC38 cells exhibited high non-specific reactivity against mock-infected cells, obscuring specific detection of viral proteins (Fig. 4). Similarly, sera from the remaining two mice (No.1 and 2) in the same group showed comparable non-specific binding to host proteins (Fig. S2).

Therefore, antibodies raised by intraperitoneal immunization with virus-infected LA795 cells could reliably detect viral proteins in IFA analysis, whereas those generated with virus-infected MC38 or LA795 cells via subcutaneous injection did not work well for IFA assay.

4. Discussion

In this study, for the first time, we identified that the BoAHV-1 can infect the mouse lung adenocarcinoma cell line LA795 and the mouse colon adenocarcinoma cell line MC38. This was demonstrated by the fact that virion-associated proteins were readily detected using two independent methods, including Western blot and immunofluorescence assay (IFA) (Figs. 1 and 2). Subsequent studies have demonstrated that the strategy of generating antibodies against viral proteins by immunizing mice with whole cells harboring the replicated virus is feasible. However, non-specific bands were also observed in the immunoblots when characterizing these antibodies using Western blot (Fig. 3). This is not surprising, as both LA795 and MC38 are murine tumor cells. The expression of oncogenes, coupled with viral infection, may alter the nature of certain host proteins, thereby allowing them to escape immune tolerance and may stimulate the production of anti-tumor antibodies, accounting for the observed non-specific bands, which remains to be elucidated in the future. Interestingly, a faint band comigrating with

Table 1
The distribution and number of the viral proteins probed at the specific molecular weights. MW, Molecular weights. No.1, 2 and 3, the mouse sera referred to as No.1, 2, and 3, produced by the specified immunization protocol for each group. +, one band with the specific molecular weight ranges. ++, two bands with the specific molecular weight ranges.

MW of probed proteins (KDa)	Immunization methods								
	IP-Virus-LA795			SC-Virus-LA795			SC-Virus-MC38		
	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
250–150					+	+			
150–100	+						++		+
100–75	+	+	+					+	+
75–55	+	++	+				+		+

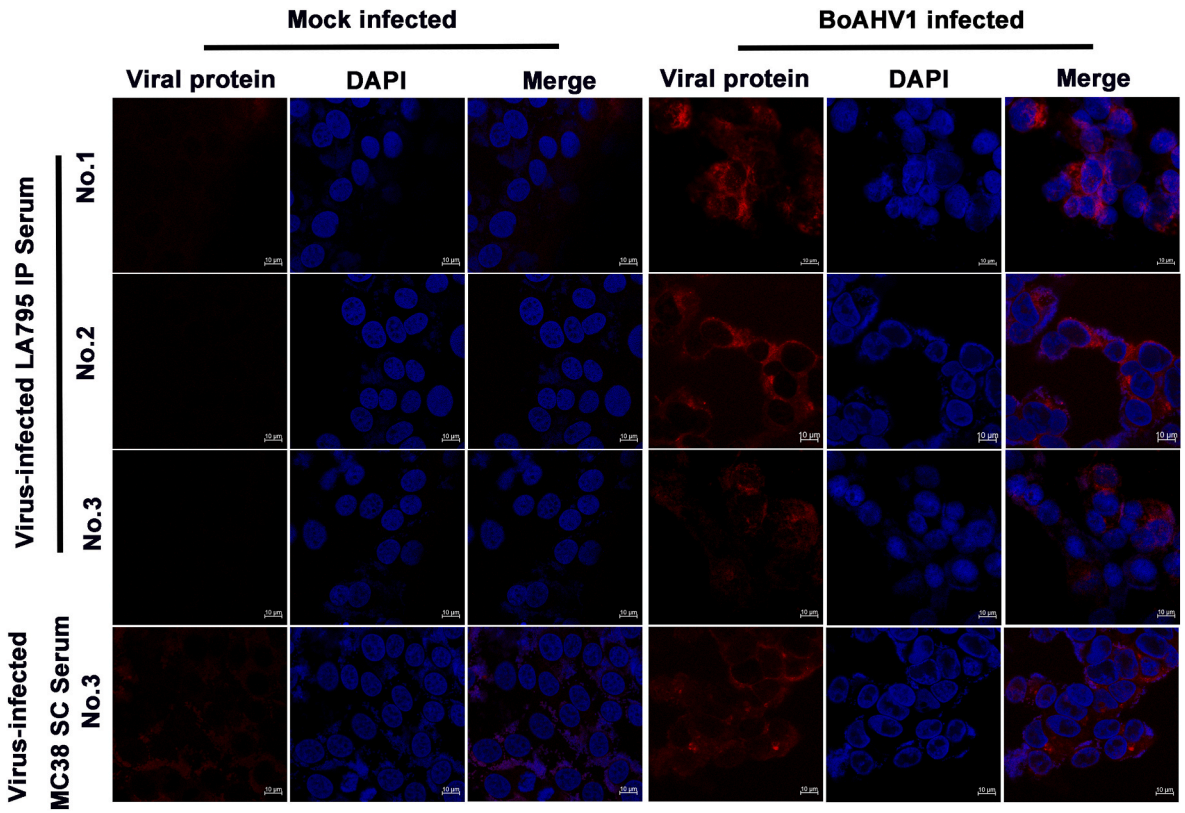

Fig. 4. Identification of the reactivation of mice sera prepared by immunization with virus-infected murine cell lines using IFA.
MDBK cells were infected with BoAHV-1 (MOI = 1) for 24 h and then immunostained via using mouse serum produced by immunization with LA795 cells through intraperitoneal injection(A) and MC38 cells via subcutaneous injection(B) with dilution of 1:500, respectively. The data shown are representative of three independent experiments. Scale bar = 10 μm.

viral proteins “c” and “h” was also detected in mock-infected cells when probed with serum from mouse No.1 and mouse No.3 immunized using virus-infected LA795 cells through intraperitoneal injections, respectively (Fig. 3A). However, similar results were not observed when the Western blot was performed using serum isolated from preimmune mouse. This may indicate that the viral protein 'c' and 'h' share homologous peptide with that of these probed host proteins, a finding implicated in revealing the virus pathogenesis, which deserve extensive studies in the future.

Immunization approaches may influence the production of antibodies, as evidenced by the serum generated from mice immunized with virus-infected LA795 cells via intraperitoneal injections, which recognized two or more viral proteins in 2 out of 3 mice (Fig. 3A). However, antibodies generated from immunizing mice with both LA795 and MC38 cells undergone virus infection via subcutaneous injections consistently recognized only one viral protein, albeit with distinct molecular weights (Fig. 3B). Notably, some mice failed to produce specific antibodies for reasons that remain unclear (Fig. 3B, No.1). It seems that immunization via intraperitoneal injections may lead to generation of antibodies targeting more viral proteins than that via subcutaneous injections. Moreover, although antibodies raised by subcutaneous immunization with virus-infected MC38 cells recognized a single viral protein on immunoblots (Fig. 3C, No.2), they failed to yield detectable staining in IFA. This discrepancy may reflect limited accessibility of the target epitope in situ, masking by cellular structures, or other as-yet-unidentified factors.

As we know, the BoAHV-1 viral genome encodes at least 73 open reading frames (ORFs)[5]. During virus productive infection in these tumor cells, viral proteins, including immediate-early (IE), early (E), and late (L) proteins, are fully expressed. More than five bands (indicative of viral proteins) could be recognized by the commercial available antibodies which are generated by immunization of goat using purified viral particles[20]. Interestingly, one to three bands could be recognized by our generated individual antibodies, respectively. The numbers of bands may vary with a mouse-specific manner (Table 1). These findings may suggestion that these probed viral proteins are immunogenic in the cell preparations, and appearance of their immunity may either largely depend on the host, or may be unusually skewed toward one immunodominant viral proteins. We noticed that the band observed at 150 kDa in the middle two panels of Fig. 3B likely corresponds to the gB glycoprotein, while the bands at 75 kDa and 55 kDa (Fig. 3A and 3C left panel) are consistent with the glycosylated and unglycosylated forms of gD, respectively. However, based on molecular-weight estimation alone is insufficient for definitive identification of a given viral protein. The essence of the viral proteins recognized by the murine serum, and the mechanism underlying the diversity of the antibodies generated by distinct mouse immunized with the same antigens are interesting issues deserving extensive studies in the future. Anyway, we provide an evidence that immunization of mice with virus-infected cells could be an efficient approach to generate monoclonal antibodies targeting a broader range of viral proteins, which bypasses the complex and time-consuming steps of producing and purifying antigens, typically required in conventional methods. Since the virus can infect bovine species, we are also interested in what will happen when we test the effectiveness of the immunization method in calves, which deserve extensive studies in the future.

This established strategy for antibody generation may also be applicable to other oncolytic viruses, such as herpes simplex virus 1 (HSV-1)[27–29], measles virus (MV)[30], reovirus type 3[31,32], adenovirus (Ads)[33,34], vaccinia virus (VACV)[35,36], myxoma virus (MYXV)[37], newcastle disease virus(NDV)[38], and Zika virus [39,40], because these viruses are also capable of infecting murine cell lines,

which warrants further investigation in the future.

Frankly, there is a risk of tumor formation when untreated tumor cells are intraperitoneally injected into mice, as certain cell lines, such as LA795, are pathogenic. However, in this study, the mice were not immunodeficient, and the cells were exposed to UV irradiation to induce damage prior to injection. Additionally, the cells were infected with BoAHV-1 at a high MOI of 10, this rendered them unhealthy and less likely to proliferate. Moreover, BoAHV-1 is an oncolytic virus, and previous studies have shown that BoAHV-1-infected tumor cells, such as MCF7 cells, exhibit dramatic reduction of tumorigenic potential *in vivo* [41]. Therefore, both viral infection and UV treatment likely contribute to minimizing the risk of tumor formation in this study. Intraperitoneal injection was restricted to a single cohort receiving virus-infected LA795 cells; no mice were given uninfected cells by this route, and only one animal received mock-infected cells subcutaneously.

Taken together, we provide a simple, rapid, and cost-effective strategy to generate BoAHV-1 antibodies by immunizing mice with virus-infected murine cell lines. This approach leverages the fact that BoAHV-1 can productively infect these tumor cells and produce viral proteins. Additionally, the mice can tolerate the presence of murine proteins without mounting an immune response, thus eliminating the need for immunization against these self-proteins. This may represent an efficient, homegrown method for generating antibodies against viral proteins.

Consent to participate

Not applicable.

Ethics approval

Animal use was approved by the Institutional Animal Care and Use Committee of Hebei University (Approval number 402,106,002) in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council.

Consent to publication

Not applicable.

Clinical trial

Not applicable.

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CRediT authorship contribution statement

Jiayu Lin: Data curation, Investigation, Methodology, Software, Validation, Visualization. **Xiaozhen Ma:** Investigation. **Naifan Zhang:** Investigation. **Yue Pang:** Funding acquisition, Investigation. **Filomena Fiorito:** Writing – review & editing. **Xiuyan Ding:** Conceptualization, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. **Liqian Zhu:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2026.102450.

Data availability

Data will be made available on request.

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