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Original Article

Mouse Fibroblast L929 Cell Line as a Useful Tool for Replication and Adaptation of Infectious Bursal Disease Virus

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Abstract

Infectious bursal disease virus (IBDV) causes a highly contagious disease associated with immunosuppression in young chickens. Production of either egg-based or primary cell-based high-quality vaccines requires time-consuming and costly procedures. To determine a suitable cell line for IBDV replication, L929 cell line was a candidate for the growth kinetics processing of the virus. The L929 cells were proliferated in monolayer, and doubling time was calculated. Replication kinetics an IBDV isolate at the multiplicity of infection 0.1 PFU/cell were determined using virus titration. To adapt IBDV on L929 cells, seven consecutive passages were performed. Virus titer and levels of apoptosis were quantitatively analyzed at each passage. The viral *VP2* gene was amplified and sequenced in three passages. An average doubling time of 21 h was estimated for monolayers of L929 cells. Although during early passages, virus growth did not produce a clear cytopathic effect (CPE), an increase in IBDV titers was observed. Serial passages led to the evidence of marked CPEs and an increase in the virus titer in the third passage. During the fourth to seventh passages, consistent CPEs characterized by the formation of granulated and round cells were evident within 24 to 48 hours post-inoculation. The titer of the virus was increased in the third passage onwards to peak in the fourth and constant at 5.9 $TCID_{50}$ until the end passage. The IBDV replication in connection with DNA fragmentation and FITC, revealed the characteristic picture of apoptosis in a time-dependent manner. We found that the IBDV could easily be adapted to L929 cells, increasing virus yields by about two orders of magnitude. These results indicated that the cell line may be useful in the production of efficient virus particles.

Keywords: Adaptation, Apoptosis, Infectious bursal disease virus, L929 cell line

1. Introduction

Infectious bursal disease (IBD) is a highly contagious and immunosuppressive disease affecting young chickens, especially between 3-6 weeks of age. The etiologic agent, IBD virus (IBDV) belongs to the genus *Avibirnavirus* of the family *Birnaviridae* has bi-segmented double-stranded RNA (dsRNA) genomes. Antigenic variants of the virus could infect the precursors of antibody-producing B cells in the bursa of Fabricius (BF) and present a wide range of pathogenic potential (1). To initiate a successful infection, the host cellular RNA binding protein Staufen1 (STAU1) recognizes the IBDV dsRNA and controls the fate of bound RNAs by involving in multiple pathways that mediate transportation of viral genomic RNA, virus replication, and immune response to virus infection (1). Similar to other non-enveloped viruses, the downstream of virus-cell membrane attachment is an endocytic entry of IBDV into the target cells. The IBDV endocytosis occurs in a stepwise manner involving attachment to the cell surface via λ light chain of surface immunoglobulin M (sIgM), membrane

perforation via Pep46 peptide, activation of signaling pathways, including the c-Jun NH2-terminal kinases (JNK) cascade, and transporting viral cargo into early endosomal compartments in a Rab5-dependent manner (2, 3). The subsequent infection triggers lymphoid depletion of B cells and the massive destruction of BF. The ability of IBDV to induce apoptosis limits the use of B lymphocyte-derived cells for vaccine production studies (4, 5).

Commonly, IBD vaccines are produced by the propagation of the virus in specific pathogen-free eggs or primary chicken embryo fibroblast (CEF) cells. Vaccine production by these platforms is labor-intensive, costly, and time-consuming, with a limited yield capacity. Different avian and mammalian cell lines have been used to grow IBDVs. Although these cell lines are manageable and reproducible, obtaining a high viral titer requires long periods of virus adaptation to cells by repeated passaging. Cultivation of viruses, especially RNA viruses, during adaptation to a cell line, can result in alterations of viral genome sequences and changes in the antigenicity of the candidate vaccine strain (6-8). An extension in the cellular receptor tropism facilitates the adaptation of the virus to cell culture. Overexpression of several proteins on the cell surface, including cytoskeleton-related proteins, enzyme-related proteins, and cell regulatory-related proteins, have been shown to be involved in the attachment and entry of the virus acting as putative receptors. The capsid protein VP2 of IBDV is responsible for the interaction with host cell receptors. Binding of the Ile-Asp-Ala (IDA) motif within the VP2 P domain as the functional ligand motif to the putative cellular receptors, such as HSP90, HSC70, annexin A2, an isoforms of chicken CD74, CD44 transmembrane glycoprotein, and α4β1 integrin can promote efficient viral infection in target cells (9-15).

In addition to these cellular factors, the pivotal involvement of surface fibroblast growth factor receptors (FGFRs) in viral entry and replication has also been emphasized for many viruses. The FGFRs, as one of the main cellular proteins, constitute a cell surface receptor tyrosine kinases family that governs various biological processes, including cell growth, survival, and apoptosis. Knock-down of FGFRs is involved in the replication of viruses via the Ras/MAPK, PI 3-kinase, and PLCg signaling pathways (16, 17). Addressing the latter impact, we examined the replication and infectivity of IBDV in L929 fibroblast cells. The cell line is established from the 95th subculture generation of the strain L derived in 1948 from a C3H/An male mouse. The cells exhibit adherent growth and are heterogeneous in morphology, including spindle-like, epithelial-like, and round shape. The replication characteristics of IBDV strains in primary CEF cells have been conducted previously. Therefore, the present study aimed to evaluate the susceptibility and adaptation procedure of the L929 cell line for the propagation of an IBDV isolate candidate for inactivated vaccine production.

2. Materials and Methods

2.1. Cell Line Culture

The mouse fibroblast L929 cells (ATCC # CCL-1) stored in the vapor phase of a liquid nitrogen tank were rapidly dissolved in a water bath at 37°C and diluted with RPMI 1640 medium (Gibco™) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in an incubator with 5% CO_2. Cells were seeded in 25-cm^2 culture flasks at 5×10^5/mL, harvested by trypsinization (0.05% trypsin), and checked for reproduction daily up to 96 h. The cells were subcultured for three passages to check the consistency of growth, confluent stage, and viability. The growth rate kinetic of L929 cells was performed for 96 h at 12-h intervals. Total cell numbers were counted in a Neubauer hemocytometer after staining with 0.4% of trypan blue, and doubling time was calculated based on the formula: $N_t=N_0\ e^{grt}$ where N_t is the number of cells at time t; N_0 is the number of cells initially at time 0; t is time; and gr is the growth rate.

2.2. Infectious Bursal Disease Virus Kinetics Replication in L929 Cell Line

A characterized IBDV isolate (accession number EU224391) propagated in the CEF cells, which is sensitive to the cytopathic effect (CPE) of IBDV, was used in this study. The virus infectious titer was calculated and presented as tissue culture infectious dose 50 ($TCID_{50}$). To screen L929 cell line for IBDV replication, cells were grown to > 80% confluency and inoculated with virus multiplicity of infection (MOI) 0.1 PFU/cell. The flasks were incubated at 37°C with adsorption for 45 min under standard conditions. The cells were washed and then covered with a fresh maintenance medium. The flasks were incubated at 37°C with 5% CO_2, and any CPE occurrence was observed at 12-h intervals up to 96 h post-inoculation (hpi) by inverted light microscopy. The IBDV replicative activity in L929 cells was assessed by titration on CEF cells carried out by three replicates.

2.3. Virus Adaptation to L929 Cell Line

To adapt the IBDV to the L929 cells, serial passages were performed. The culture of each passage with about 80% CPE was subjected to freeze-thaw cycles twice. Cell debris was removed by a light clarifying centrifugation (3,000 rpm for 10 min), and the supernatant was used for further passages up to the 7th passage level. At 24-h intervals, the cell culture was assessed for i) CPE development, ii) virus titration, iii) apoptosis induction, and iv) molecular characterization of IBDV adapted in L929 cell.

2.4. Assessment of Apoptosis

Apoptotic L929 cells after getting infected by IBDV, were determined using DNA fragmentation (5). Apart from this assay, Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, USA) was used to quantitatively measure cell death. Briefly, the IBDV-inoculated cells were harvested at 24, 48, 72, and 96 hpi through trypsinization, washed twice with cold PBS, and stained with FITC Annexin V and PI for 15 min in the dark at room temperature. The binding buffer was subsequently added to the Annexin V-FITC sample and analyzed in BD Accuri™ C6 Plus flow cytometer. The un-inoculated cell culture was considered as mock control.

2.5. Molecular Detection by RT-PCR

Virus RNA was extracted from the harvested cell culture using the easy-BLUE™ Total RNA Extraction Kit. The extracted RNA was subjected to Maxime™ RT-PCR PreMix Kit for a one-step RT-PCR assay to amplify the *VP2* gene (18). The amplified PCR product was stained with RedSafe and visualized using 1.0% agarose gel electrophoresis for the expected band. All the kits and reagents were purchased from iNtRON Biotechnology, Korea. To evaluate the impact of adaptation of IBDV to L929 cells, the molecular features of the viral *VP2* gene in serial cell passages were characterized by comparative sequence analysis. The nucleotide and the deduced amino acid sequences of the gene in passages P2, P5, and P7 were detected and compared to the virus seed using ClustalW.

2.6. Statistical Analysis

Statistical data were obtained using the SPSS statistical software (version 20). The Student's t-test was used to determine the statistical significance. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

Based on the findings of the present research, the L929 cells grew rapidly, producing confluent monolayers. The adherent cells displayed a spindle shape and epithelial-like morphology. The cell growth behaved similarly, with no statistical differences in cell viability after three continuous passages. More than 90% of the total population of cells had their viability until 72 h of the cell passage. The time required for cells to reach the confluence stage was normal. An average doubling time of 21 h was estimated based on the growth rate (0.033 cells/h) and time duration of 96 h.

According to the primary scheme, the susceptibility of L929 cells for IBDV propagation was evaluated. The IBDV-inoculated cells did not exhibit marked CPEs

during the cultivation of the virus. The virus infectivity to L929 cells was low at 24 hpi and showed a titer of 2.8 $TCID_{50}$/mL that gradually increased to 3.2 over time. The result suggests that L929 can be a permissive cell for IBDV infection and support the virus life cycle.

During the adaptation of IBDV to the L929 cells, the virus titer increased and remained unchanged after four passages. The mean viral titers increased significantly from 3.1 to 5.9 $TCID_{50}$ after four passages (Figure 1). Despite the increase in virus titers, we failed to achieve higher values of IBDV titers in the late passages. In addition to the increase in infectious titer, the morphological changes in the cell monolayer became more evident. Based on the microscopic analysis, appearance of CPE in L929 cells started about 24 h after IBDV inoculation in the 3rd passage. The predominant CPE was an initial rounding followed by a detachment of the cells at 72 hpi (Figure 2). The maximum lesion of the cells amounted to about 90% in the late passages, and the destructive changes were usually most apparent after the 4th passage. Moreover, the duration for CPE occurrence decreased to 48 hpi.

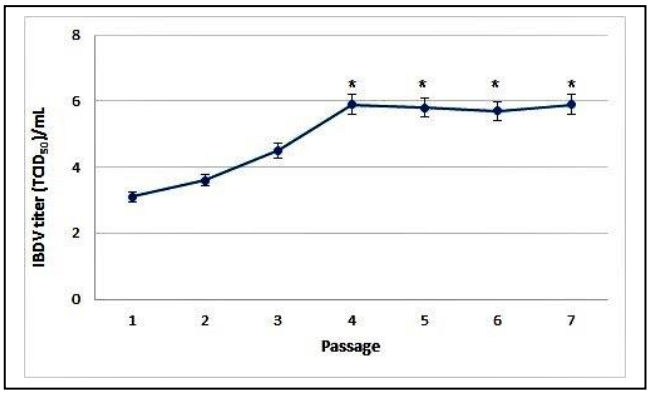

Figure 1. The replication kinetics of infectious bursal disease virus during adaptation to L929 cells. Results are presented as the mean viral titers of three independent experiments and error bars indicate the standard deviation of the mean. Student's t-test was performed to determine the *P* value; **P*<0.05

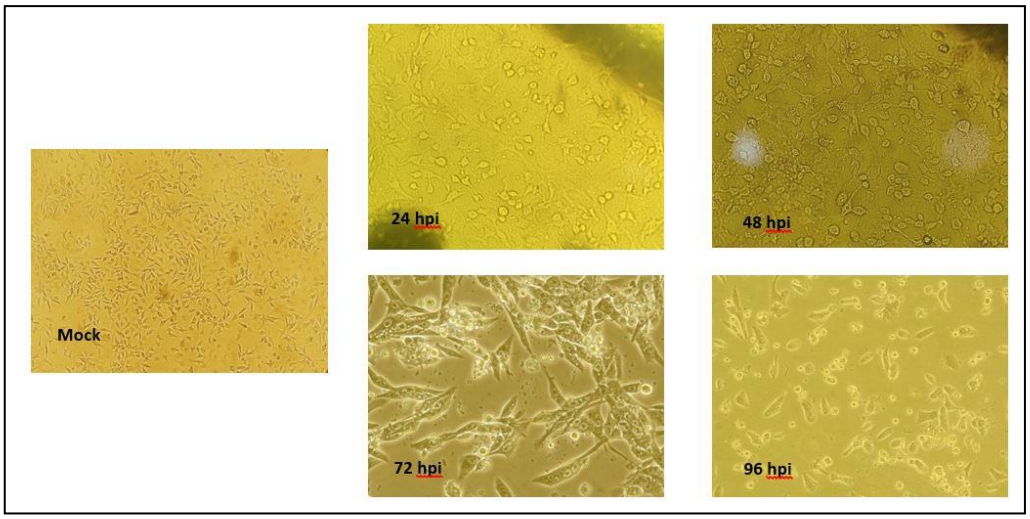

Figure 2. Infectious bursal disease virus adaptation on L929 cell line. Development of CPE is exemplarily shown at 4th passage compared to the un-inoculated (Mock) cells

The characteristic apoptotic laddering of DNA started at 48 hpi with weak bands, and a complete DNA laddering was observed at the end hours, especially for the late passages (Figure 3). The cells presented preliminary DNA fragmentation at 48 hpi indicating the cells could be in a state of late apoptosis.

To further confirm the IBDV-induced apoptosis, L929 apoptotic cells were detected by flow cytometry after Annexin V-FITC and PI dual staining. The IBDV infection led to the induction of apoptosis in L929 cells, compared with uninfected control cells. The percentages of apoptotic cells were significantly increased (P<0.05) in a time-dependent manner (Figure 4).

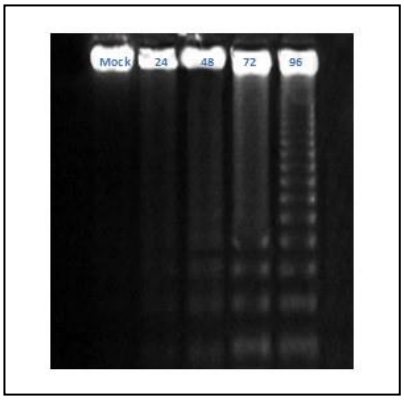

Figure 3. The IBDV-infected L929 cells were undergo apoptosis. The gel demonstrates the time course of DNA fragmentation. Major laddering is a late event in apoptosis

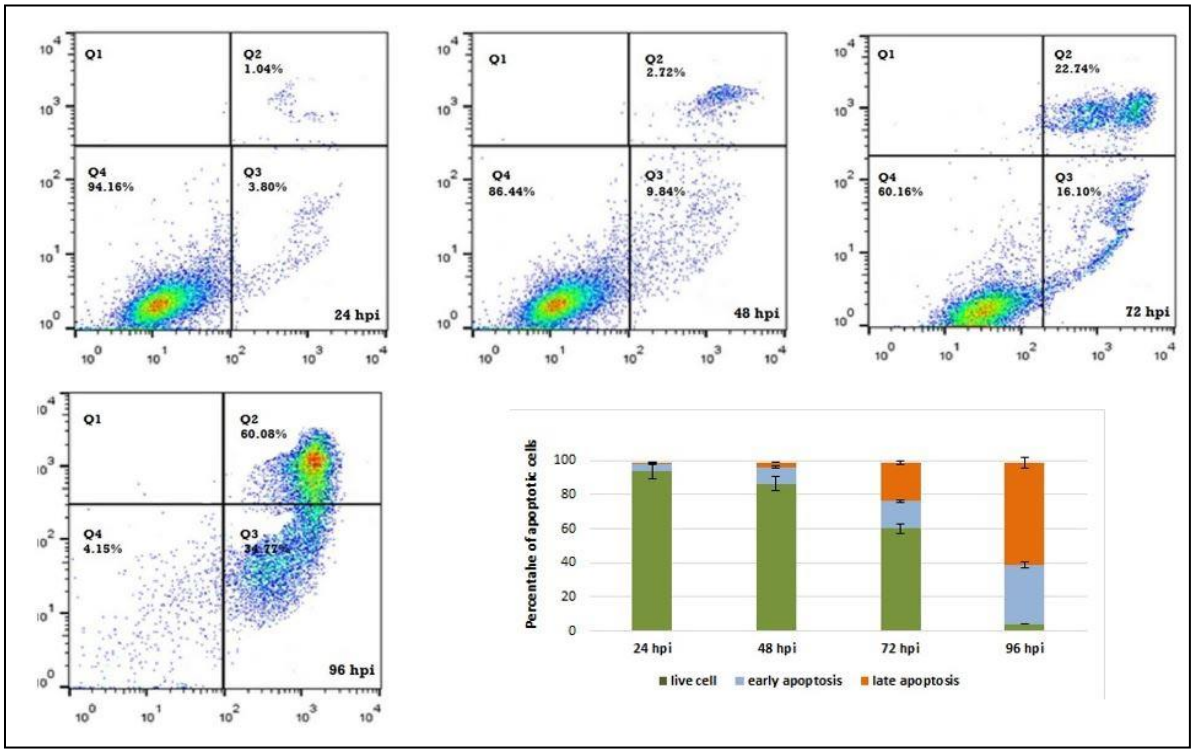

Figure 4. Cell apoptosis/necrosis of the IBDV-infected L929 cells stained with annexin V-FITC in conjugation with propidium iodide at different hpi. Each density plot quadrant shows: Q1; necrotic cells (annexin− PI+), Q2; late apoptotic cells (annexin+ PI+), Q3; early apoptotic cells (annexin+ PI−), and Q4; viable cells (annexin− PI−). The graph data show the promotion of early and late apoptosis (Q2 and Q3) in L929 cells. The bar indicates a significant difference (P < 0.05)

A peak apoptotic rate of 60.08% was observed at 96 hpi by an increase in the proportion of the cells in the late apoptosis (upper-right quadrant in figure 4). The proportion of apoptotic cells population was similar, especially for the late passages, and about 5% of cells were found to be apoptotic at 24 hpi, which increased to more than 12% at 48 hpi. Then, the percentages of apoptotic cells significantly increased with an increasing viral infection time. Virus titration revealed that the majority of L929 cells were not apoptotic early after the infection. The apoptotic cells were appeared at later days in response to IBDV infection. The time-dependent replication of IBDV in L929 cells revealed that the virus titer increased quickly, and the replication rate gradually slowed down after 48 hpi, consistent with the increase in apoptotic cell population.

The *VP2* genes from IBDV-infected cell cultures at the different passages were amplified at about 1,277 bp and sequenced at three passages. The nucleotide and the deduced amino acid sequences of the gene passaged various times in L929 cells were analyzed and compared with the parental virus genome sequence. Only two nucleotide substitutions were found at the 5th passage, which did not lead to amino acid codon change.

4. Discussion

Previous studies indicated that several mammalian continuous cell lines are susceptible to the IBDV infection and support virus replication, even at a low infectivity rate. However, the production of distinct and visible CPEs, as well as high virus titers, was frequently not reported in these cells. The majority of field IBDV isolates do not replicate in these cells without prior adaptation. Likewise, the adaptation of an isolate to propagate in cell culture is laborious and time-consuming. Selective pressures during the adaption, dynamics of viral population kinetics, and quality of culture media and supplements are the main factors to be taken into account in using the cell culture system (19). In this study, we used the L929 cell line to reduce the risks and costs of the IBDV adaptation.

In the first part of the study, we examined the ability of the fibroblastic cell for virus replication. Based on the gradually increased virus titer, L929 cells were permissive to IBDV; however, they did not show marked CPEs before adaptation. Several fibroblast and epithelial cell lines are reported to be susceptible to IBDV propagation (7, 8, 20, 21). It can be speculated that IBDV binding to the cells is not mediated only by SIgM, and the co-expressed cellular proteins certainly act as receptors during virus attachment (19). Viruses usually choose a large variety of coreceptors based on availability, functional affinity, and ability to trigger cellular signaling pathways that involve viral replication rate. Distribution of the receptor mainly specifies the permissiveness of cell for virus in simulating infection *in vitro*. However, viruses usually bind to multiple receptors to enhance the binding affinity in case of a lack of the specific receptor. Based on the IBDV-host interactions, VP2 protein binds to different cellular factors and forms a subviral particle (SVP) which plays an essential role in cell tropism determination and the host cellular receptors interactions. Among VP2 domains, the P domain located at 206 to 350 amino acid residues is responsible for the binding of SVPs with cellular receptors (22). Previous studies showed HSP90 and HSC70 expresses on the surface of the chicken DF-1 cell line, an immortalized cell line derived from primary CEF. The IBDV uses these ubiquitous molecular chaperones as the putative cellular receptor complex for entry into DF-1 cells (9, 15). Viral proteins are generally dependent on chaperone function for folding. Despite the involvement of HCS70 in clathrin-mediated endocytosis or HSP90 in association with membrane lipid rafts at the early stage of virus entry, the specific role of these proteins in IBDV binding has been unclear. Following attachment, viruses activate the signaling systems of the cell for endocytic uptake. Annexin II cellular protein implicates in the infection of several viruses by involving in the regulation of the endocytotic pathway. It has been shown that the binding of IBDV to Annexin II is effective for the

promotion of virus infection in DF-1 cells (12). In an alternative binding approach, integrins act as receptors for viruses that either display a short peptide Arg-Gly-Asp (RGD) sequence on their capsid or do not depend on RGD. Although it is not clear that IBDV uses RGD-dependent integrins, it is known that the virus activates intracellular-signaling cascades (23). Typically, the signaling pathways involve the activation of integrins and receptor tyrosine kinases followed by the activation of PI3Ks, Src kinases, and membrane fission factors downstream. In the case of IBDV, the virus activates the phosphorylation of c-Src in the α4β1 integrin-mediated pathway manner. Then, the virus entry is induced by activating downstream PI3K/Akt-RhoA signaling and actin cytoskeleton rearrangement (24). Many viruses utilize FGFRs either for physical attachment to the cell surface or stimulating receptor tyrosine kinase signaling. Activation of the intracellular protein kinase domain initiates downstream signaling cascades, including MAPK, STAT, and PI3K/Akt pathways, eventually leading to viral entry and infection (14, 23). It has not yet been proved that FGFRs are involved in IBDV entry and replication, knowledge about the activation PI3K/Akt-RhoA signaling during IBDV infection constitutes a logical basis to choose the L929 cell line for replication of this virus. The cross-talk between FGFRs and other cell receptors on the surface of L929 cells, effectively promoted IBDV replication rate and infectivity.

In the second part of our study, IBDV adaptation in L929 cells was determined following subsequent passages. Production characterized CPEs, including granulation, rounding, and finally, detachment of cells was seen after three passages in L929 cells. The viral kinetics were quantified via $TCID_{50}$ and FITC assays. The virus titer remained at a constant level up to the second passage, then raised to more than 5. A plateau was reached at the fourth passage, and in the later passages, the viral titers remained around 5.3 $TCID_{50}$. The timing and penetrance of apoptotic cells in relationship with IBDV replication was examined. The FITC detected a small population of apoptotic cells at 24 hpi. A positive correlation between the proportion of apoptotic cells and viral titer was observed in the IBDV-infected cells. Previous studies have shown that induction of apoptosis in a variety of cell lines is mediated by interacting VP2 with ORAOV1 (3, 4). Shutting off the cellular protein synthesis by VP2 results in the impairment of ROS levels in IBDV-infected cells. The accumulated ROS levels are associated with the activation of the PKR-dependent apoptosis pathway (3, 25). Moreover, expression of the viral VP3 and VP5 has been related to the regulation of the IBDV-induced apoptotic response early during IBDV infection. The VP3 acts as an anti-apoptotic molecule by inhibiting PKR phosphorylation to prevent VP2-induced apoptosis. The anti-apoptotic activity of VP5 is dependent on binding to PI3K to facilitate virus dissemination in the cells (26, 27). It has been shown that a single point mutation in the VP2 P domain affects SVP cell binding and virus infectivity (22, 28). Therefore, we detected the identity of IBDV during adaptation to L929 cells, and no amino acid variations were detected in VP2 sequenced in passages P2, P5, and P7 compared to the virus seed.

Our findings illustrate the potential of the L929 cell line to support the replication of IBDV. Expression of multiple receptors on the surface of L929 cells enhances the binding affinity and promotes virus propagation. Whether this is related to the interactions between multiple cell receptors and virus requires further studies. Quantifying both viral yield and apoptosis of IBDV-infected L929 cells in subsequent passages could help in a detailed analysis of the replication dynamics of the virus. This continuous cell line makes many parallel analyses in the virus replication possible and reduces the use of embryonated eggs or primary chicken-origin cells in the IBDV vaccine production; however, the experiment should be completed with other IBDV strains.

Authors' Contribution

S. S. (the corresponding author) designed the study; S. T. carried out the analysis and interpretation of data with contributions from all co-authors; all authors administrated the technical and material support and participated in acquisition of data; S. S. drafted the manuscript and all authors read and approved the final the manuscript.

Ethics

This study as a non-interventional experiment is not included human or animal subjects.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Li J, Zheng S. Role of MicroRNAs in host defense against infectious bursal disease virus (IBDV) infection: a hidden front line. Viruses. 2020;12(5):543.
2. Gimenez MC, Rodríguez Aguirre JF, Colombo MI, Delgui LR. Infectious bursal disease virus uptake involves macropinocytosis and trafficking to early endosomes in a R ab5-dependent manner. Cell Microbiol. 2015;17(7):988-1007.
3. Qin Y, Zheng SJ. Infectious bursal disease virus-host interactions: multifunctional viral proteins that perform multiple and differing jobs. Int J Mol Sci. 2017;18(1):161.
4. Qin Y, Xu Z, Wang Y, Li X, Cao H, Zheng S. VP2 of infectious bursal disease virus induces apoptosis via triggering oral cancer overexpressed 1 (ORAOV1) protein degradation. Front Microbiol. 2017;8:1351.
5. Shahsavandi S, Ebrahimi MM, Sadeghi K, Mahravani H. Apoptotic response of chicken embryonic fibroblast cells to infectious bursal disease virus infections reflects viral pathogenicity. In Vitro Cell Dev Biol-Anim. 2014;50(9):858-64.
6. Lawal Nu, Hair-Bejo M, Arshad SS, Omar AR, Ideris A. Adaptation and molecular characterization of two malaysian very virulent infectious bursal disease virus isolates adapted in BGM-70 cell line. Adv Virol. 2017;2017.
7. Luo J, Zhang H, Teng M, Fan J-M, You L-M, Xiao Z-J, et al. Surface IgM on DT40 cells may be a component of the putative receptor complex responsible for the binding of infectious bursal disease virus. Avian Pathol. 2010;39(5):359-65.
8. Rekha K, Sivasubramanian C, Chung I-M, Thiruvengadam M. Growth and replication of infectious bursal disease virus in the DF-1 cell line and chicken embryo fibroblasts. BioMed Res Int. 2014;2014.
9. Lin T-W, Lo C-W, Lai S-Y, Fan R-J, Lo C-J, Chou Y-m, et al. Chicken heat shock protein 90 is a component of the putative cellular receptor complex of infectious bursal disease virus. J Virol. 2007;81(16):8730-41.
10. Liu A, Pan Q, Li Y, Yan N, Wang J, Yang B, et al. Identification of chicken CD74 as a novel cellular attachment receptor for infectious bursal disease virus in bursa B lymphocytes. J Virol. 2020;94(2):e01712-19.
11. Liu A, Pan Q, Wang S, Zhang Y, Li Y, Wang Y, et al. Identification of chicken CD44 as a novel B lymphocyte receptor for infectious bursal disease virus. J Virol. 2022;96(6):e00113-22.
12. Ren X, Zhang L, Gao Y, Gao H, Wang Y, Liu C, et al. Binding chicken Anx2 is beneficial for infection with infectious bursal disease virus. Virus Res. 2015;210:232-40.
13. Schneider-Schaulies J. Cellular receptors for viruses: links to tropism and pathogenesis. J Gen Virol. 2000;81(6):1413-29.
14. Zhu LQ, Wu SL, Zhang GP, Zhu GQ. The cellular receptors for infectious bursal disease virus. Afr J Biotechnol. 2008;7(25).
15. Chen C, Qin Y, Qian K, Shao H, Ye J, Qin A. HSC70 is required for infectious bursal disease virus (IBDV) infection in DF-1 cells. Virol J. 2020;17(1):1-11.
16. Maddaluno L, Urwyler C, Rauschendorfer T, Meyer M, Stefanova D, Spörri R, et al. Antagonism of interferon signaling by fibroblast growth factors promotes viral replication. EMBO Mol Med. 2020;12(9):e11793.
17. Kumar R, Khandelwal N, Thachamvally R, Tripathi BN, Barua S, Kashyap SK, et al. Role of MAPK/MNK1 signaling in virus replication. Virus Res. 2018;253:48-61.
18. Ebrahimi MM, Shahsavandi S, Shayan P, Goudarzi H, Masoudi S. Recombinant VP2 expressed in baculovirus and adjuvanted with TIR-TLR7: a vaccine candidate

against infectious bursal disease virus. Comp Clin Path. 2018;27:911-6.
19. Genzel Y. Designing cell lines for viral vaccine production: Where do we stand?. Biotechnol J 2015;10(5):728-40.
20. Jackwood DH, Saif Y, Hughes J. Replication of infectious bursal disease virus in continuous cell lines. Avian Dis.1987:370-5.
21. Yip CW, Hon CC, Zeng F, Leung FCC. Cell culture-adapted IBDV uses endocytosis for entry in DF-1 chicken embryonic fibroblasts. Virus Res. 2012;165(1):9-16.
22. Letzel T, Coulibaly F, Rey FA, Delmas B, Jagt E, Van Loon AA, et al. Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. J Virol. 2007;81(23):12827-35.
23. Delgui L, Ona A, Gutierrez S, Luque D, Navarro A, Caston JR, et al. The capsid protein of infectious bursal disease virus contains a functional α4β1 integrin ligand motif. Virology. 2009;386(2):360-72.
24. Ye C, Han X, Yu Z, Zhang E, Wang L, Liu H. Infectious bursal disease virus activates c-Src to promote α4β1 integrin-dependent viral entry by modulating the downstream Akt-RhoA GTPase-actin rearrangement cascade. J Virol. 2017;91(3):e01891-16.
25. Shahsavandi S, Ebrahimi MM, Ghadiri MB, Samiee MR. Tween 80 improves the infectivity of BCL1 cell-adapted infectious bursal disease virus. J Virol Methods. 2022;304:114502.
26. Rodríguez-Lecompte JC, Niño-Fong R, Lopez A, Markham RF, Kibenge FS. Infectious bursal disease virus (IBDV) induces apoptosis in chicken B cells. Comp Immunol Microbiol Infect Dis. 2005;28(4):321-37.
27. Wei L, Hou L, Zhu S, Wang J, Zhou J, Liu J. Infectious bursal disease virus activates the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway by interaction of VP5 protein with the p85α subunit of PI3K. Virology. 2011;417(1):211-20.
28. Qi X, Zhang L, Chen Y, Gao L, Wu G, Qin L, et al. Mutations of residues 249 and 256 in VP2 are involved in the replication and virulence of infectious bursal disease virus. PloS One. 2013;8(7):e70982.