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Critical role of cholesterol in bovine herpesvirus type 1 infection of MDBK cells

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ABSTRACT

Cholesterol is involved in the life cycle of many viruses. Here, we examined the role of cholesterol for both viral envelope and target cell membrane for bovine herpesvirus type 1 (BoHV-1) infection. Cholesterol depletion by pretreatment of Madin–Darby bovine kidney (MDBK) cells with a cholesterol-sequestering drug methyl-β-cyclodextrin (MβCD), inhibited the production of BoHV-1 in a dose-dependent manner. This inhibitory effect was partially reversed by cholesterol replenishment, indicating that the reduction was caused by cholesterol depletion. Cholesterol depletion at the post-entry stage only had a mild effect on the virus production. However, cell membrane cholesterol depletion did not reduce the virus attachment. In addition, treatment of BoHV-1 particles with MβCD also reduced the virus infectivity significantly and the effect was partially reversed by addition of exogenous cholesterol. Taken together, these data implicated that cell membrane cholesterol mainly contributed to BoHV-1 entry into MDBK cells and the viral envelope cholesterol was also essential for the virus infectivity.

1. Introduction

BoHV-1 is an alphaherpesvirus causing abortion, respiratory, and genital infections in cattle (Tikoo et al., 1995; Straub, 2001). For the latent infection mainly established in ganglion cells and tonsils, it becomes a reservoir of BoHV-1 allowing the virus spreading to susceptible animals (Winkler et al., 2000). Now it is worldwide distributed and tends to be endemic in most populations, though national and regional variations occur (Kampa et al., 2009).

Lipid rafts refer to putative microdomains distributed in the membrane, which are similar to the rafts floating in the sea. It is thought that lipid rafts are enriched in cholesterol, glycosphingolipids, sphingomyelin, phospholipids with long, unsaturated acyl chains, glycosylphosphatidylinositol (GPI)-linked proteins and at least some membrane-spanning proteins (Simons and van Meer, 1988; Simons and Ikonen, 1997; Simons and Toomre, 2000). Approximately 15–20% of the plasma membrane surface area is believed to consist of lipid rafts (Parolini et al., 1999; Schutz et al., 2000). The tight packaging domains are associated with numerous important biological processes, such as transmembrane signal transduction, apoptosis, cell adhesion, migration, synaptic transmission, organization of the cytoskeleton, and protein sorting during endocytosis and exocytosis (Brown and London, 1998; Simons and Toomre, 2000; Harris and Siu, 2002; Tsui-Pierchala et al., 2002). However, the extraction of cholesterol by various chemicals destroys the raft organization and consequently blocks biological processes that depend on lipid rafts. So the function of lipid rafts in some biological process can be evaluated by studying the role of cholesterol.

Accumulating evidence suggests that many pathogens, especially viruses require cholesterol at multiple stages of

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their lifecycles. Human immunodeficiency virus type-1 infection requires cholesterol, both in the target cell membrane and the viral envelope (Guyader et al., 2002; Liao et al., 2001, 2003). Many viruses require cholesterol in the target cell membrane, such as Semliki Forest virus (Phalen and Kielian, 1991; Ahn et al., 2002), severe acute respiratory syndrome-coronavirus (Li et al., 2007) and SV40 virus (Anderson et al., 1996). For influenza virus and duck hepatitis B virus, the presence of cholesterol in its viral envelope is critical, but it is not essential in the target cell (Sun and Whittaker, 2003; Funk et al., 2008). Whereas numerous strains of flavivirus dengue virus and yellow fever virus 17D enter and infect cells independent of cholesterol (Umashankar et al., 2008).

In this study, we sought to clarify the importance of cholesterol at cell membrane and viral envelope for BoHV-1 infection of MDBK cells. We found that intact cell membrane cholesterol was indispensable for the virus entry and the viral envelope cholesterol was also imperative for the virus infectivity.

2. Materials and methods

2.1. Cells and virus

MDBK cells were maintained in DMEM (Gibco BRL) supplemented with 10% horse serum, and passaged whenever they became confluent. The Colorado 1 strain of BoHV-1 was used for this study (Wang et al., 2003). Viruses were propagated in MDBK cells as previously described (Lawrence et al., 1986), except that the horse serum was used in place of calf serum. The collected culture medium from infected MDBK cells was titrated and stored at −80 °C.

2.2. Antibodies and reagents

Bovine sera against BoHV-1 was raised by the infection of animals, experimentally (kindly provided by Dr. Fei Xue, Harbin Veterinary Research Institute, China). Goat anti-bovine immunoglobulin secondary antibody coupled to fluorescein isothiocyanate (FITC) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), methyl-β-cyclodextrin (MβCD), cholesterol and fluorescent cholera toxin B subunit (CTB-FITC) were bought from Sigma. The reagents are water soluble.

2.3. Plaque formation assay

The process of plaque formation assay was followed as previously described with some modification (Dasika and Letchworth, 1999). In brief, the monolayer cells in 24-well plates were infected with 200 μl diluted virus and incubated for indicated times at 37 °C, the inoculum was removed and overlaid with DMEM supplemented with 1% horse serum and 0.5% agarose. The cytopathic effect of BoHV-1 is very evident such as causing the infected cells enlarged or ballooned and then become rounded, take on a refractile appearance. So the plaques were directly counted under microscope after 48 h incubation at 37 °C, when the CPE was clearly evident.

2.4. Assays for cell viability and cholesterol level

Cell viability was assessed by the MTT assay as previously described with some modification. (Guo et al., 2007). Briefly, MDBK cells were seeded in 96-well microplates at 1×10^4 cells/well. Eight replicates were mock treated or treated with MβCD at various concentrations of 2.5, 5, 10, 15 and 20 mM for 30 min at 37 °C in a humidified atmosphere of 5% CO_2. After further incubation in a final volume of 200 μl DMEM for 48 h, 30 μl MTT solution (2 mg/ml in PBS) was added to each well. The cells were then incubated at 37 °C for 4 h, the supernatant was removed and 150 μl of DMSO was added to each well to solubilize the formazan. Then, the microplate was shaken on a rotary platform for 10 min. Finally, the absorbance value was measured at wave length of 550 nm using a Wellscan (Labsystems, Santa Fe, NM, USA). The mean optical density of the cell control wells was assigned a value of 100%.

Efficiency of cholesterol removal by MβCD was assessed with flow cytometry assay. MDBK cells seeded in six-well plates for three replicates were mock treated or treated with 2.5, 5, 10 and 20 mM MβCD for 30 min at 37 °C. After washing three times with ice-cold PBS, the detached cells were incubated with CTB-FITC at 10 μg/ml in serum-free DMEM for 30 min on ice. Flow cytometry was carried out using a Becton–Dickinson FACSCalibur flow cytometer with 10,000 events collected and data were analysed using FlowJo software, version 7.1.3.

2.5. Effect of the cell membrane cholesterol depletion on the virus entry

To determine if the cholesterol depletion at the virus entry stage affects the virus replication, the monolayer cells seeded in 24-well plates were mock treated or treated with MβCD at the concentrations of 2.5, 5, 10, 20 mM for 30 min at 37 °C before incubation for 1 h with BoHV-1 of $50TCID_{50}$. After treatment with citrate buffer (40 mM citric acid, 10 mM KCl, 135 mM NaCl, pH 3.0) for exactly 1 min to inactivate cell membrane bound but unpenetrated virions as described elsewhere (Chung et al., 2005; Highlander et al., 1987), the cells were further incubated to determine the virus yield with plaque formation assay.

For cholesterol replenishment, monolayer of MDBK cells in 24-well plates mock pretreated or pretreated with 10 mM MβCD for 30 min at 37 °C, were supplemented or mock supplemented with 400 μg/ml cholesterol in DMEM and incubated for 1 h at 37 °C (Li et al., 2007). After washing, the cells were subjected to BoHV-1 infection, followed by the treatment with citric buffer, the virus yield was determined with plaque formation assay.

2.6. Effect of cell membrane cholesterol depletion at the post-entry stage on the virus replication

To analyze whether the depletion of cholesterol after virus entry affects virus replication, MDBK cells seeded in 24-well plates were pre-incubated for 1 h with BoHV-1 of $50TCID_{50}$. Following the treatment with citrate buffer they were treated with 10 mM MβCD for 30 min at 37 °C