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Integrity of foot-and-mouth disease virions bound to the cells

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Summary. – In this study, we examined the integrity of Foot-and-mouth disease virus (FMDV) particles during their binding to the surface of BHK-21 cells under physiological condition. For monitoring of the virus integrity we used blocking of the endocytosis with dynasore and cytochalasin D followed by RT-PCR for viral protein VP1 and the resistance of FMDV to the treatment of RNase A. Our results showed that integrin binding to VP1 did not cause a substantial conformational change in the viral capsid. Furthermore, treatment with RNase A showed no effect on the infectivity of intact as well as cell-bound virions. Our findings confirmed that FMDV entered the host cells in the form of intact virions.

Keywords: Foot-and-mouth disease virus; virus-cell binding; RNase A; RT-PCR; VP1 protein

Introduction

The protein shell encasing virus particles known as the capsid consists of several oligomeric structural protein subunits. The capsid encloses the genome of the virus not only protecting it from digestion by DNase, RNase, and other protein enzymes, but also supplying the attachment (receptor-binding) sites through which the virus interacts with specific homologous receptors on host cells triggering virion entry (Sieczkarski *et al*., 2005; Marsh *et al*., 2006). The stability of viral capsid is critical for the protecting of viral genome, especially when virions are released from the host cell and enter the blood, tissue fluids or lymph fluids (Baxt *et al*., 1980; Kunkel *et al*., 2002). Infectivity of the virus particles is dependent on the functional integrity of both viral genome and capsid, Structural changes in the capsid may diminish viral genome stability or affect the attachment to the host cell receptors directly by jeopardizing the viral infectivity (Seth, 1994; Pietiainen *et al*., 2002). The intact virus particle is significantly more resistant to the RNase digestion than unprotected RNA genome (Bonville *et al*., 2003; Tafech *et al*., 2006).

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Abbreviations: FMDV = Foot-and-mouth disease virus

Interaction of enteroviruses (the family *Picornaviridae*) with their receptors causes a structural rearrangement of the virions resulting in the release of VP4 protein and externalization of the N-terminal extension of VP1 protein. The 135 S virus particle is further degraded to the 80 S particle by interaction with the membranes and RNA genome is released (Bayer *et al*., 2001; Burman *et al*., 2006; Belnap *et al*., 2007). FMDV is also a member of the *Picornaviridae* family with an RNA genome core surrounded by the capsid consisting of four protein subunits (VP1-VP4). Infection by FMDV is mediated by the host cell integrins interacting with the arginine-glycine-aspartate tripeptide motif of the capsid protein VP1 (Burman *et al*., 2006). In general, picornaviruses utilize clathrin-mediated endocytic pathways to enter host cells (Scodeller *et al*.,1984; O'Donnell *et al*., 2005; Abban et al., 2008), and at least four different integrin receptors are utilized (Baxt *et al*., 1980; DeTulleo *et al*., 1998).

Most studies investigating virus-host cell interactions to date have been performed using human cell lines, such as the breast epithelial cell line MCF-10A, since almost all of the reagents available to detect cellular structures react only with the human cells (Fricks *et al*., 1990; Pulli *et al*., 1997). Another method is to use soluble recombinant integrins to analyze virion integrity, but this method is unable to provide information about three-dimensional interactions between the virions and various integrins on the cell surface (Lehmann *et al*., 2005). Because FMDV is one of the small-

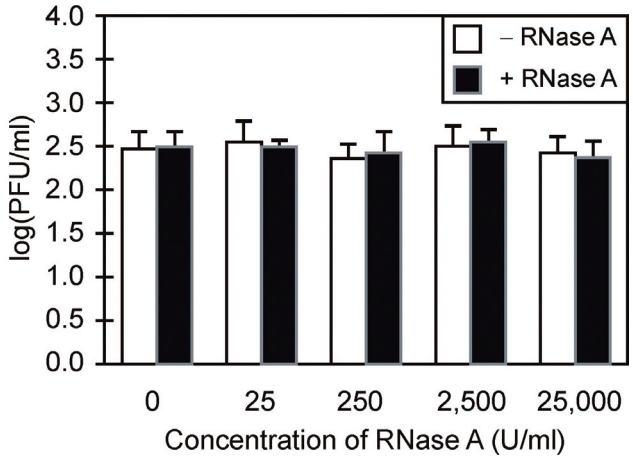

Fig. 1

Effect of RNase on intact FMDV virions

Different concentrations of RNase A (axis x) and amount of PFU (axis y).

est viruses with a diameter of 25 nm, slight changes in the virus capsid are difficult to reveal by the electron microscopy (Jackson *et al*., 2003; Dicara *et al*., 2007).

In this study, we examined the integrity of FMDV virions bound to the surface of BHK-21 cells under physiological conditions and blocked them from subsequent endocytosis

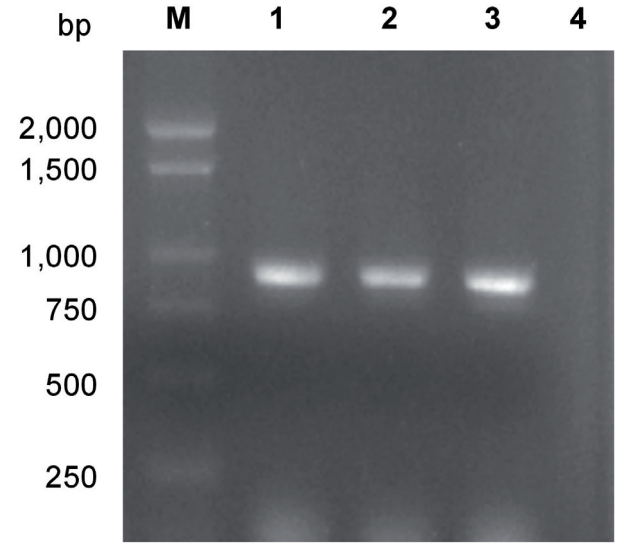

Fig. 2

Effect of RNase on FMDV virions bound to cells

Intact virions in PBS (lane 1); virions bound to cells (lane 2); virions bound to cells treated with RNase A (lane 3); PBS (lane 4); DNA size markers (lane M).

with dynasore and cytochalasin D. We used an RT-PCR for viral protein VP1 and resistance to RNase A as the markers of virion integrity. The results showed that RNase A had no effect on the intact as well as cell-bound virions indicating that FMDV entered host cells in the form of intact virions.

Materials and Methods

Cells and virus titration. FMDV O strain HKN/2002 was isolated from the infectious vesicular fluid of an infected pig. Viral stocks were grown in BHK-21 cells that were maintained in DMEM containing 10% FCS (Invitrogen). A virus titer of FMDV strain HKN/2002 was determined by the plaque assay. A series of ten-fold virus dilutions were inoculated (0.5 ml) onto a confluent monolayer of BHK-21 cells in a six-well plate to determine the number of PFU in viral samples.

RNase A digestion assay. 3×10^3 PFUs of virus in the medium (50 µl) were treated with different concentrations of RNase A in PBS at 37°C for 1 hr. After treatment, the virulence of virus was evaluated using the plaque assay with BHK-21 cells as described above.

Virus-cell binding. 1 mmol/l of dynasore and 5 mmol/l of cytochalasin D (both Sigma-Aldrich) were added to the blank control wells and wells containing 70% confluent BHK-21 cells in six-well plates. Cells were incubated for 30 mins to inhibit the endocytosis process. Then, 2×10^7 PFU of FMDV virions were added and incubated along with 250 U/ml of RNase A for 1 hr.

RT-PCR for VP1. QIAamp viral RNA mini kit (Qiagen) was used to extract the viral RNA from virus suspensions after RNase A digestion according to the manufacturer's directions. 50 U of RNase inhibitor (PerkinElmer) were then added to each RNA sample to inhibit the activity of RNase A. RT-PCR was used to amplify the VP1 DNA segment using the primers 5'-CCGGAAT TCATGACCACCTCTGCGG-3' (F) and 5'-CGCGGATCCTCAA GAAGCTGTTTG-3' (R) designed against the FMDV HKN/2002 VP1 gene sequence.

Results

Effect of RNase A on the intact virions

Our results showed that there was no significant difference in the number of PFU, when FMDV was treated with the different concentrations of RNase A indicating that RNase A does not affect the infection rate of FMDV (Fig. 1).

Effect of RNase A on the virions bound to cells

To determine whether the interaction of virions and integrins on the cell surface could induce structural changes in the viral capsid under physiological conditions, we used

dynasore and cytochalasin D to completely stop the process of virus endocytosis. Virions bound to the integrins on the cell surface were digested by RNase A. After digestion, the genomic RNA was extracted and RT-PCR was carried out to amplify VP1 gene. The results showed that the interaction between virions and receptors did not induce any structural changes in the viral capsid (Fig. 2).

Discussion

Data relating to the interaction of virions and integrins are limited, especially picornaviral receptor-dependent conformational alterations that occur during the process of internalization and uncoating (Duque *et al*., 2004). Some soluble integrins exhibited little binding capability to FMDV, even though the soluble integrins were able to bind to their natural ligand, vironectin. However, this did not result in the generation of any conformational changes in the virions (O'Donnell *et al*., 2005).

In this study, we investigated whether the interaction between cell receptors and FMDV virions under physiological conditions could result in the structural changes of capsid. We halted the virion endocytosis process using two endocytosis inhibitors dynasore and cytochalasin D. Dynasore is the inhibitor of dynamins, a family of GTPases that facilitate the budding of clathrin-coated pits leading to the formation of coated vesicles. Cytochalasin D is the cell-permeable potent inhibitor of actin polymerization, which disrupts actin microfilaments and inhibits the pathway of endocytosis inside the host cells (Anderson *et al*., 1996; Bayer *et al*., 2001). In our experimental setting the virions were fixed on the cell surface. The results showed that three-dimensional interaction of virions and various integrins did not change the structural stability of capsid and FMDV was structurally unaffected by the interaction with its receptor in cell culture medium. Taken together, these findings confirmed that FMDV utilizes the host integrins as receptors, enters host cells as an intact virion by the clathrin-mediated endocytosis pathway with the release of viral genome in the cytoplasm of host cells.

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